

ATGAAGGTCTCCGTGGCTGCCCTCTCCTGCCTCATGCTTGTACTGCCCTGGATCCCAG 60  
M K V S V A A L S C L M L V T A L G S Q

GCCCCGGGTCAAAAAGATGCAGAGACAGAGTTCAATGATGTCAAAGCTTCATTGGAAAAT 120  
A R V T K D A E T E F M M S K L P L E N

CCAGTACTTCTGGACAGATTCCATGCTACTAGTGCTGACTGCTGCATCTCCACACCCCA 180  
P V L L D R F H A T S A D C C I S Y T P

CGAACGCATCCCGTGTTCACTCCTGGAGAGTTACTTTGAAACGAACAGCGAGTGCTCCAAG 240  
R S I P C S L L E S Y F E T N S E C S K

CCGGGTGTCACTTCTCACCAAGAAGGGGGCACGTTCTGTGCCAACCCAGTGATAAG 300  
P G V I F L T K K G R R F C A N P S D K

CAAGTTCAAGTTGCATGAGAATGCTGAAGCTGGACACACGGATCAAGACCAGGAAGAAT 360  
Q V Q V C M R M L K L D T R I K T R K N

TGA 363

\*

FIG.1

ATGAAGATCTCCGTGGCTGCAATTCCCTCTTCCCTCATCACCATGCCCTAGGGACC  
M K I S V A A I P F F L L I T I A L G T

AAGACTGAATCCTCCTCACGGGACCTTACCAACCCCTCAGAGTGCTGCTCACCTACACT  
K T E S S S R G P Y H P S E C C F T Y T

ACCTACAAGATCCCGCGTCAGCGGATTATGGATTACTATGAGACCAACAGCCAGTGCTCC  
T Y K I P R Q R I M D Y Y E T N S Q C S

AAGCCCAGAATTGTCTTCATCACCAAAGGGGCCATTCCGTCTGTACCAACCCAGTGAC  
K P G I V F I T K R G H S V C T N P S D

AAGTGGGTCAAGGACTATATCAAGGACATGAAGGAGAACTGA  
K W V Q D Y I K D M K E N \*

FIG.2

1 ATGAAGGGCTTGCAGCTGCCCTCCTGTCCTCGTCTGCACCATGGCCCTCTGCTCCTGT 60  
M K G L A A A L L V L V C T M A L C S C

61 GCACAAGTTGGTACCAACAAAGAGCTCTGCTGCCCTCGTCTATACCTCCGGCAGATTCCA 120  
A Q V G T N K E L C C L V Y T S W Q I P

121 CAAAAGTTCATAGTTGACTATTCTGAAACCAGCCCCAGTGCCCCAAGCCAGGTGTCACTC 180  
Q K F I V D Y S E T S P Q C P K P G V I

181 CTCCCTAACCAAGAGAGGCCGGCAGATCTGTGCTGACCCAATAAGAAGTGGGTCCAGAAA 240  
L L T K R G R Q I C A D P N K K W V Q K

241 TACATCAGCGACCTGAAGCTGAATGCCTGA 270  
Y I S D L K L N A \*

FIG.3

CK $\beta$ -8	MKVSVAAALSCMLVTALGSQARVTKDAETEFMMSKLPLENPVLLDRFHAT	50
	.. : .   ..  ...  :	
MIP-1 $\alpha$	MQVSTAALAVLLCTMALCNQFSASLAAD.....T	29
CK $\beta$ -8	SADCCISYTPRSIPCSLLESYFETNSECSPGVIFLTGGRRFCANPSDK	100
	...   :   .    ..::    .  :       :  .   :   .	
MIP-1 $\alpha$	PTACCFSYTSRQIPQNFIADYFETSSQCSPGVIFLTKRSRQVCADPSEE	79
CK $\beta$ -8	QVQVCMRMLKLDTRIKTRKN	120
	::  .  .	
MIP-1 $\alpha$	WVQKYVSDLELSA	92

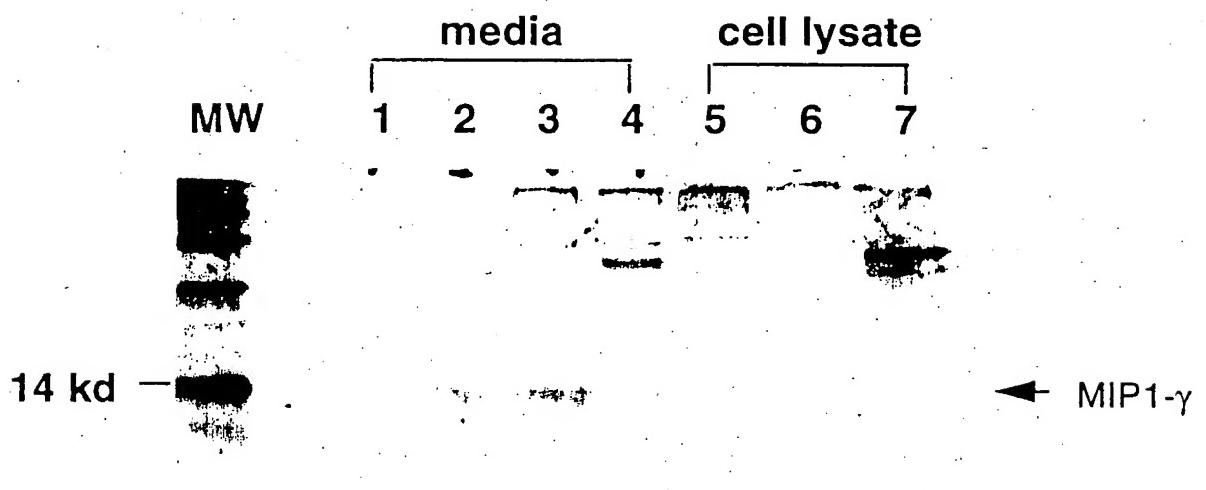
**FIG.4**

1 MKGLAAALLVLVCTMALC....SCAQVTNKELCCLVYTSWQIPQKFIVD 46  
| . . ||| ||:||||| | . : . . . |||:|||:|||:|||.||.|||  
1 MQVSTAALAVLLCTMALCNQVLSAPLAADTPTACCFSYTSRQIPQNFIAD 50  
  
17 YSETSPQCPKPGVILLTKRGRQICADPNKKWVQKYISDLKLNA 89  
| ||| .||.|||:|||:|||||||:|||...|||:|||:|||.|||  
51 YFETSSQCSKPSVIFLT K RGRQVCADPSEEWVQKYVSDLELSA 93

FIG.5

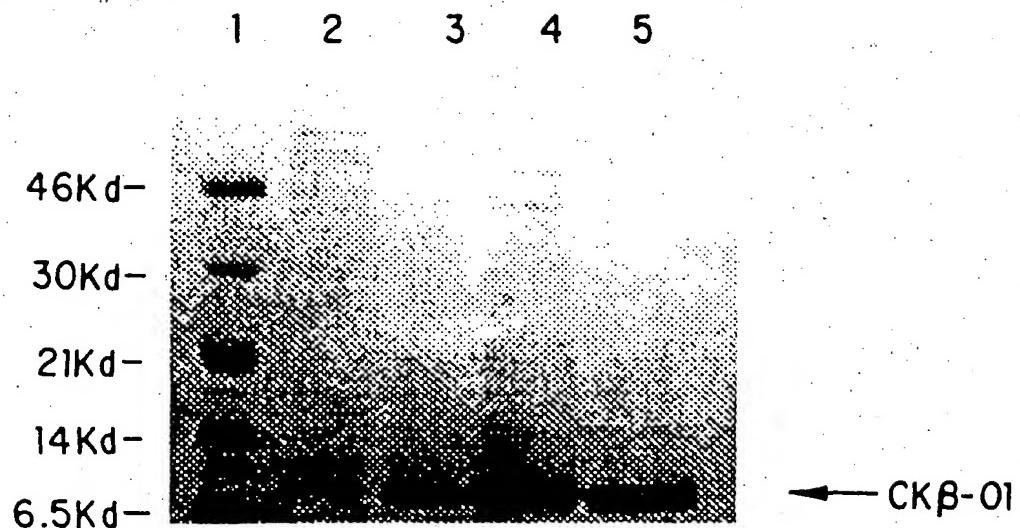
CKβ-1	MKISVAAIPFLLITIALGKTTESSSRGPYHPSECCFTTYKIPRQRIM	50
	.: . .: .   :  ... . .: ..   :  .   ..	
MIP-1α	MQVSTAALA.VLLCTMALCNQF.SASLAADTPTACCFSYTSRQIQPQNFIA	48
CKβ-1	DYYETNSQCSKPGIVFITKRGHSVCTNPSDKWVQDYIKDMKEN	94
	:  .      :  :   ::  .   :   ..  .   .	
MIP-1α	DYFETSSQCSKPGVIFLTKRSRQVCADPSEEWQKYVSDLESA	93

## FIG. 6



1 = mock, 2 and 3 = MIP1- $\gamma$ -HA, 4 = I $\kappa$ B-HA  
5 = mock, 6 = MIP1- $\gamma$ -HA, 7 = I $\kappa$ B-HA

**FIG. 7**



LANE #	SAMPLE
1	LOW MW MARKERS
2	CK $\beta$ -1 BACULOVIRUS SUPERNATANT
3	HEPARIN COLUMN PURIFIED CK $\beta$ -1
4	S/M COLUMN PURIFIED CK $\beta$ -1
5	HW50 PURIFIED CK $\beta$ -1

FIG. 8

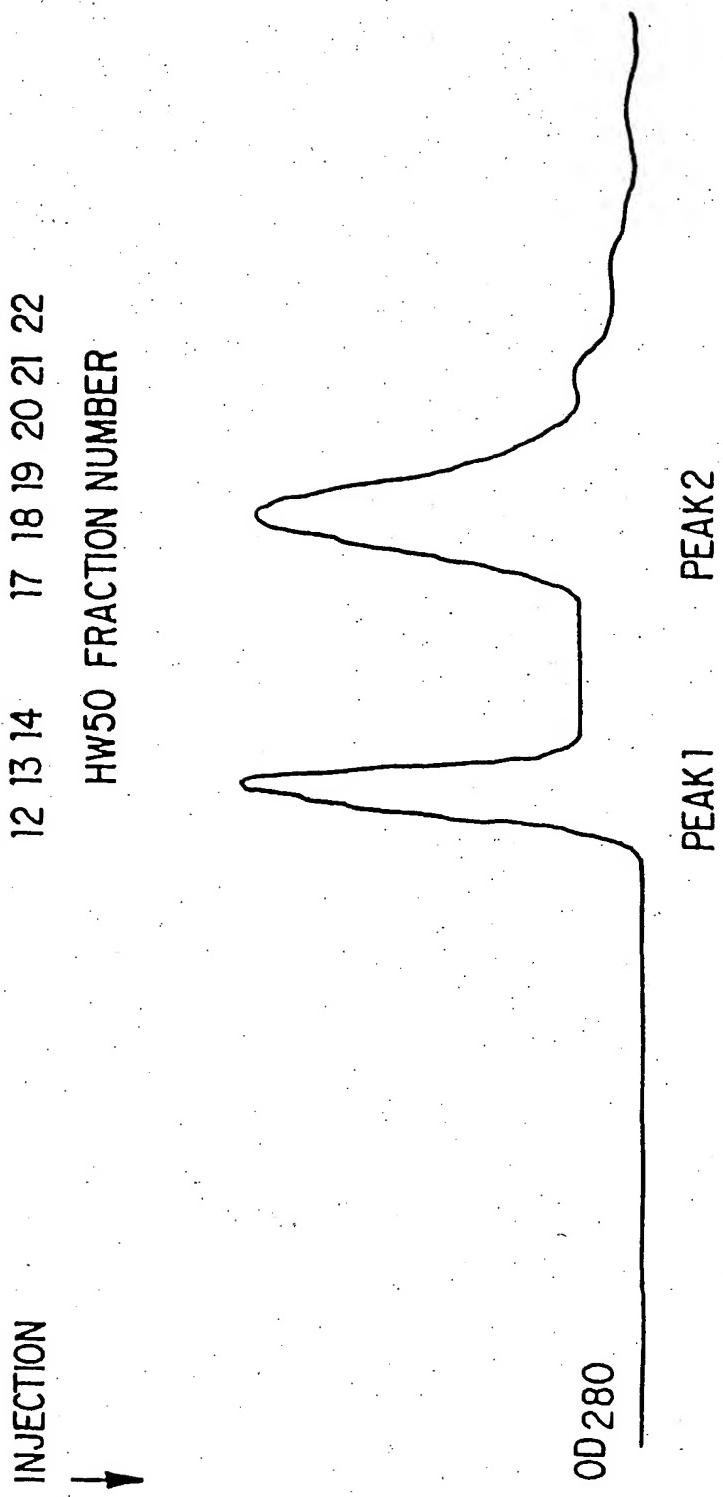
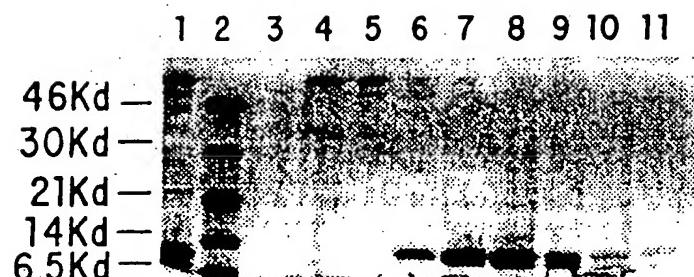


FIG. 9A



PEAK1    PEAK2

LANE#    SAMPLE

1	HW50 LOAD
2	LOW MW MARKERS
3	HW50 FRACTION 12
4	13
5	14
6	17
7	18
8	19
9	20
10	21
11	22

FIG. 9B

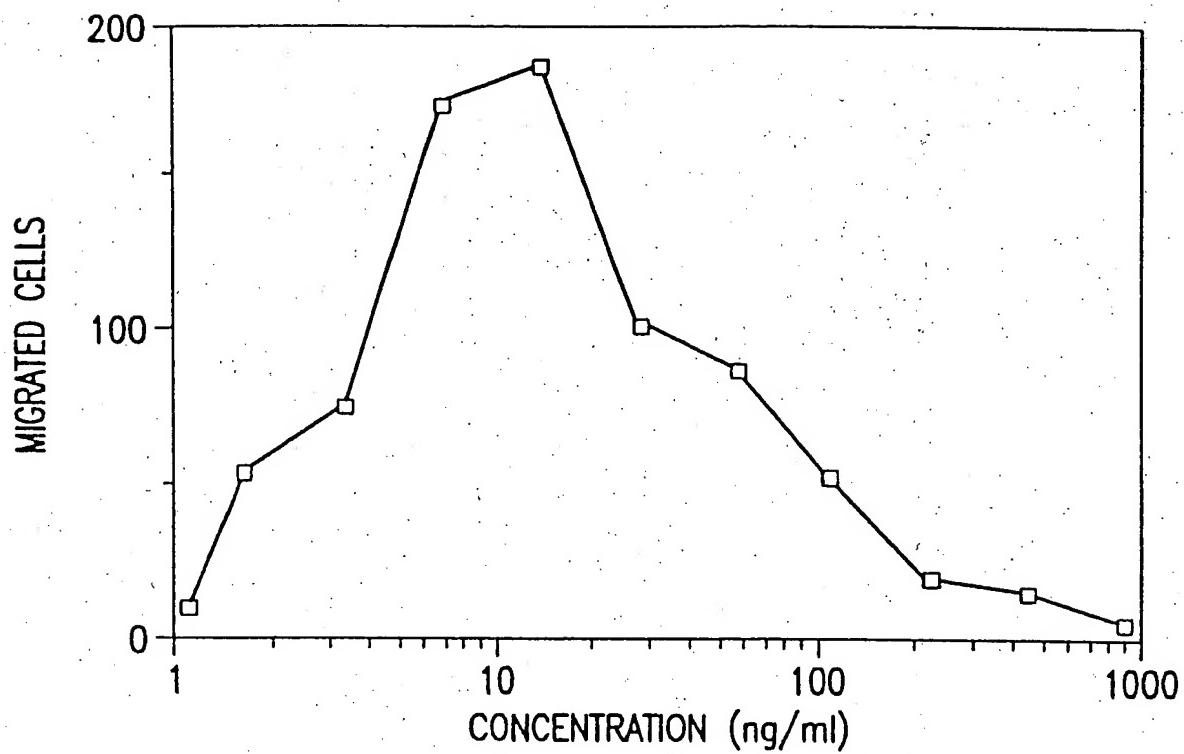


FIG.10A

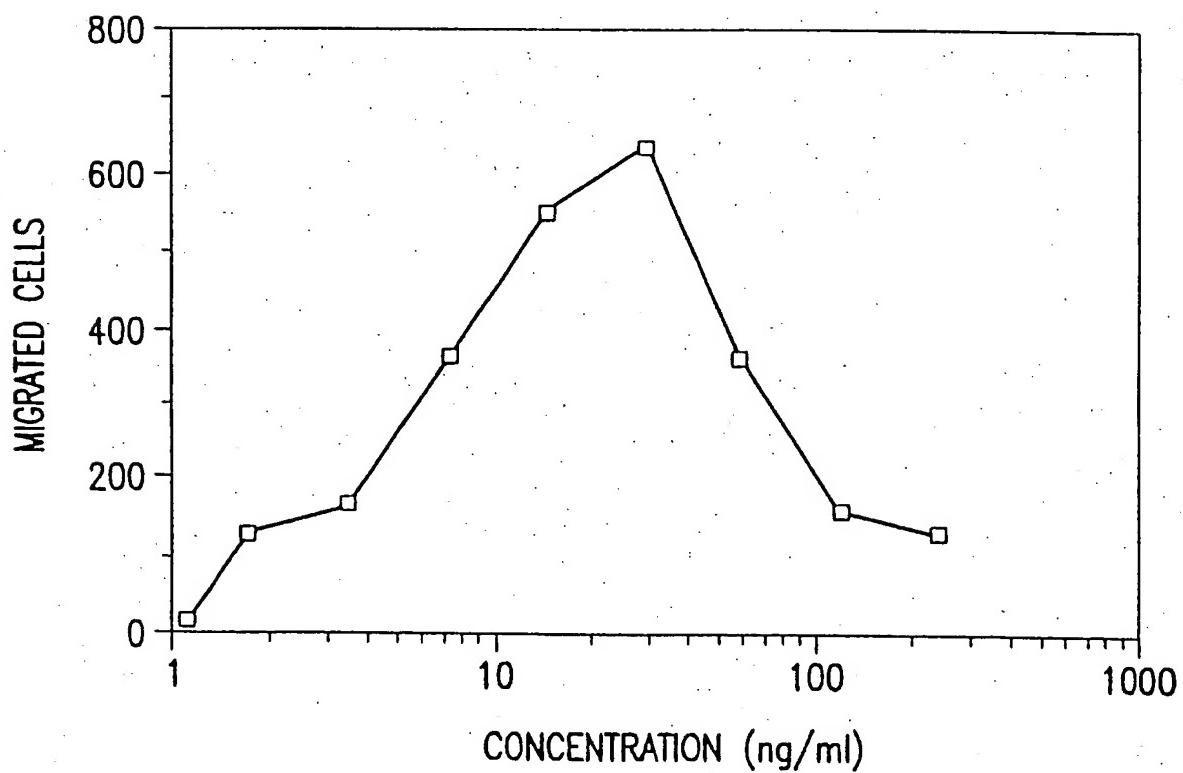


FIG.10B

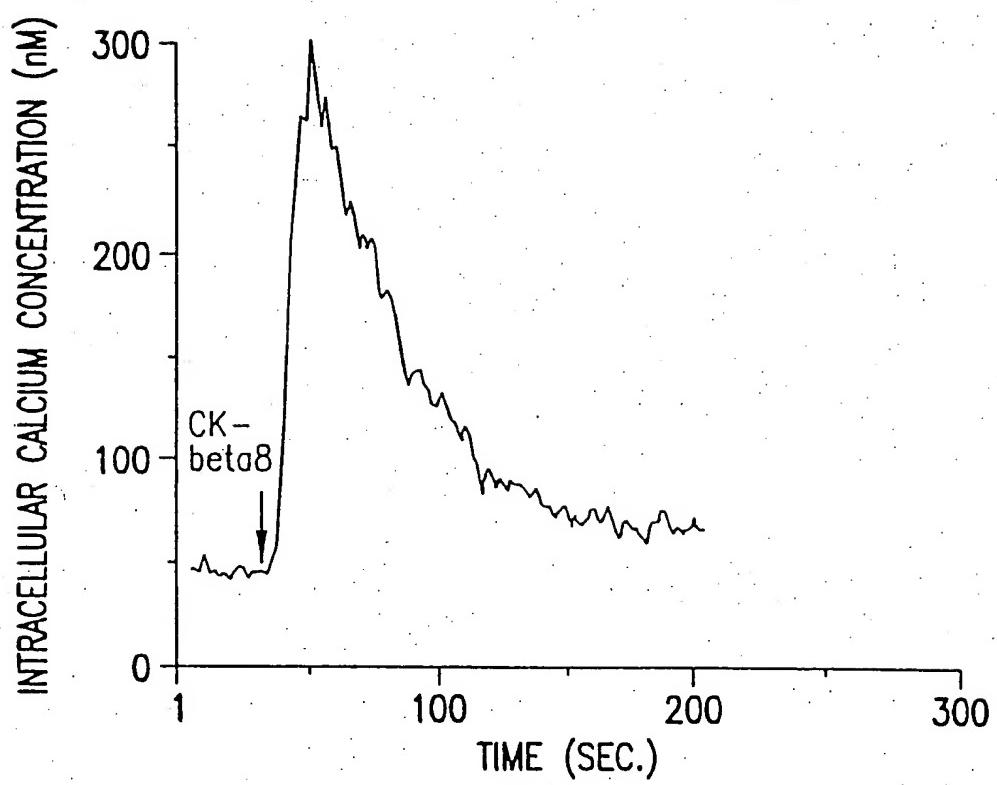


FIG.11

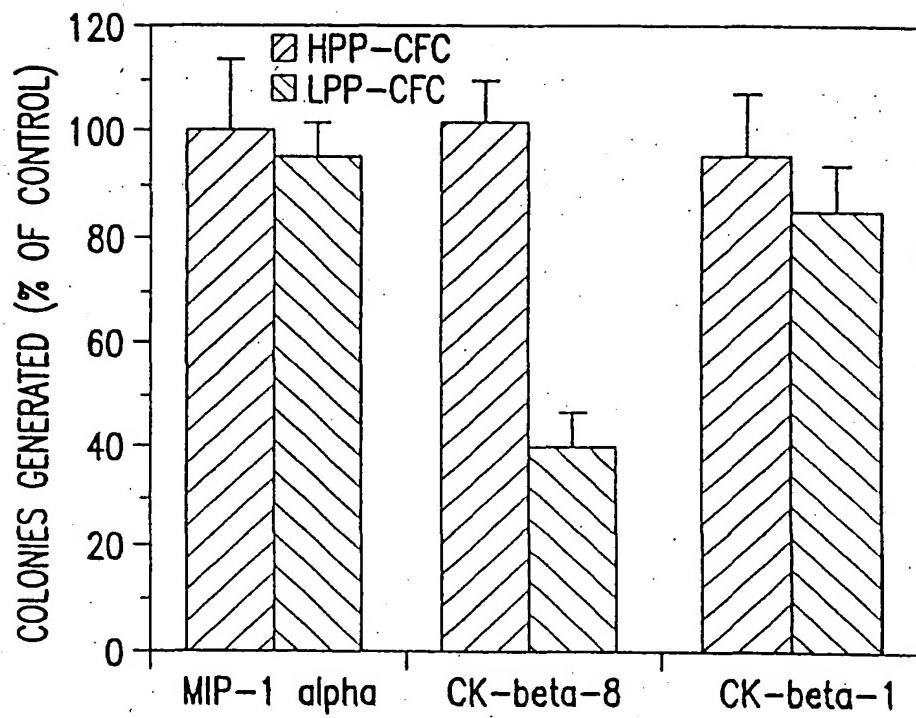


FIG.12

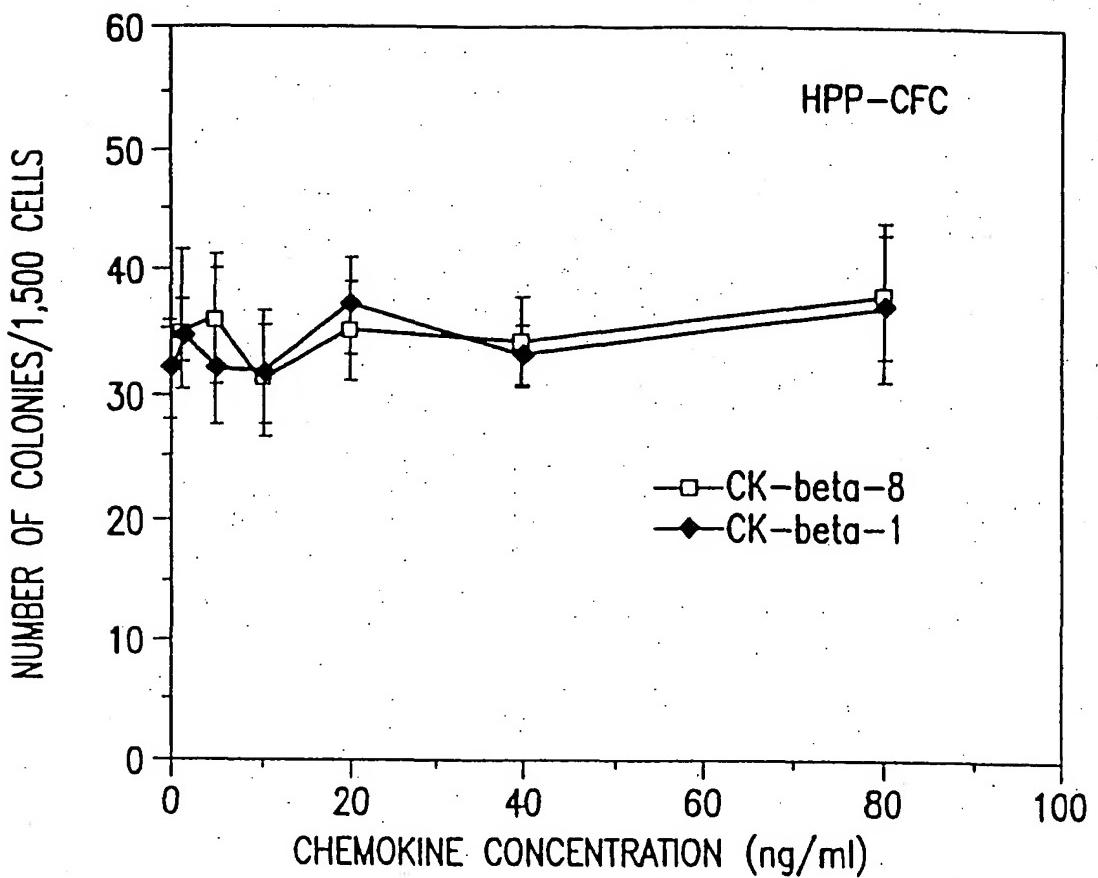


FIG.13A

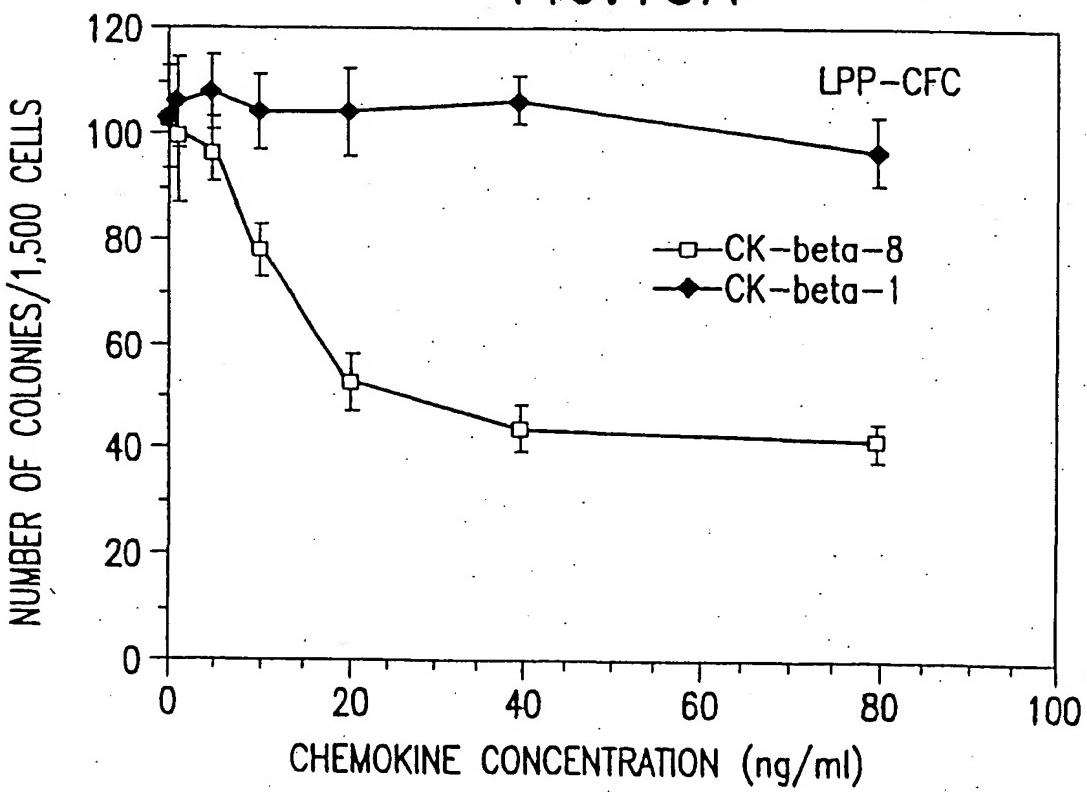
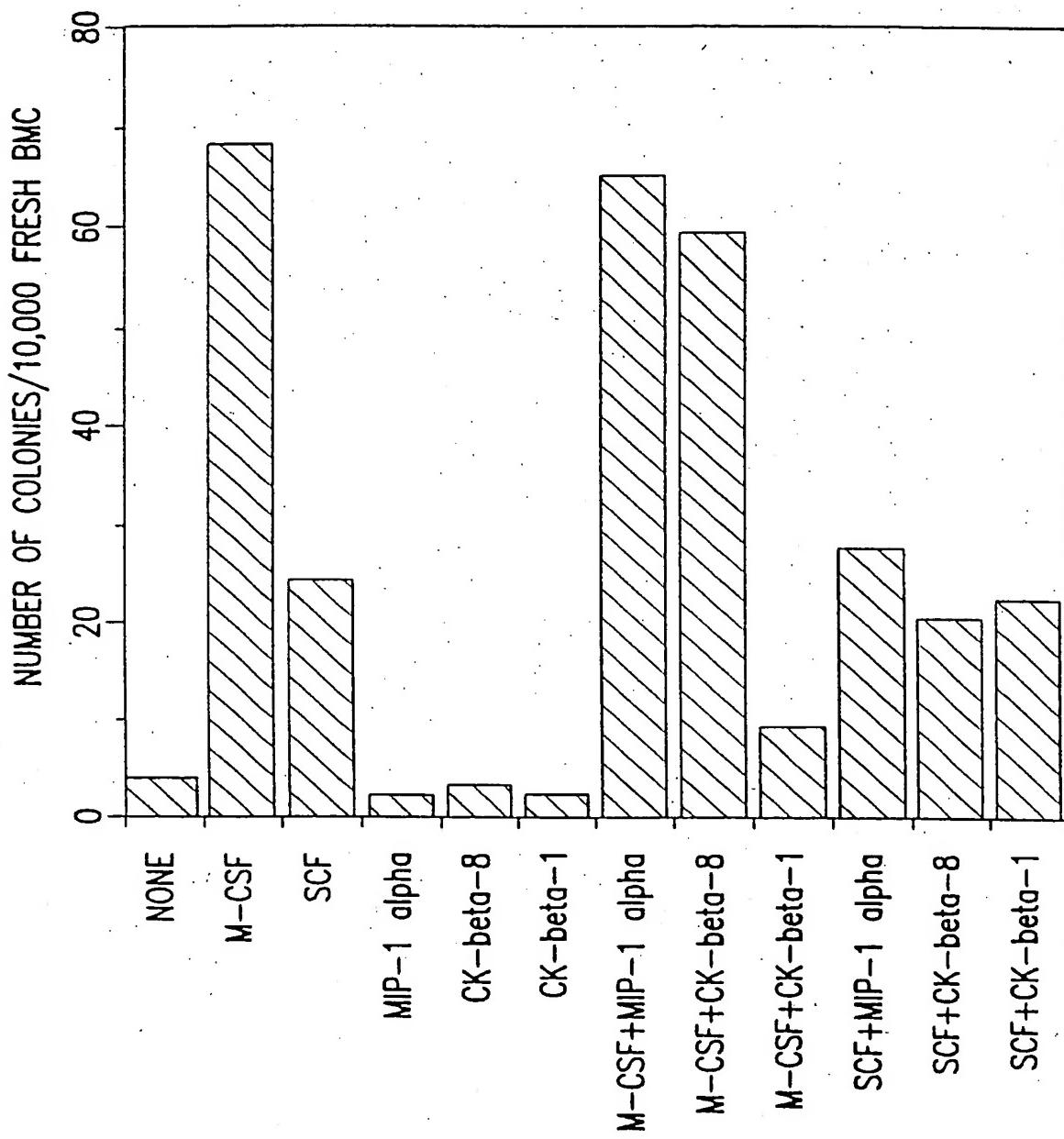


FIG.13B

FIG. 14



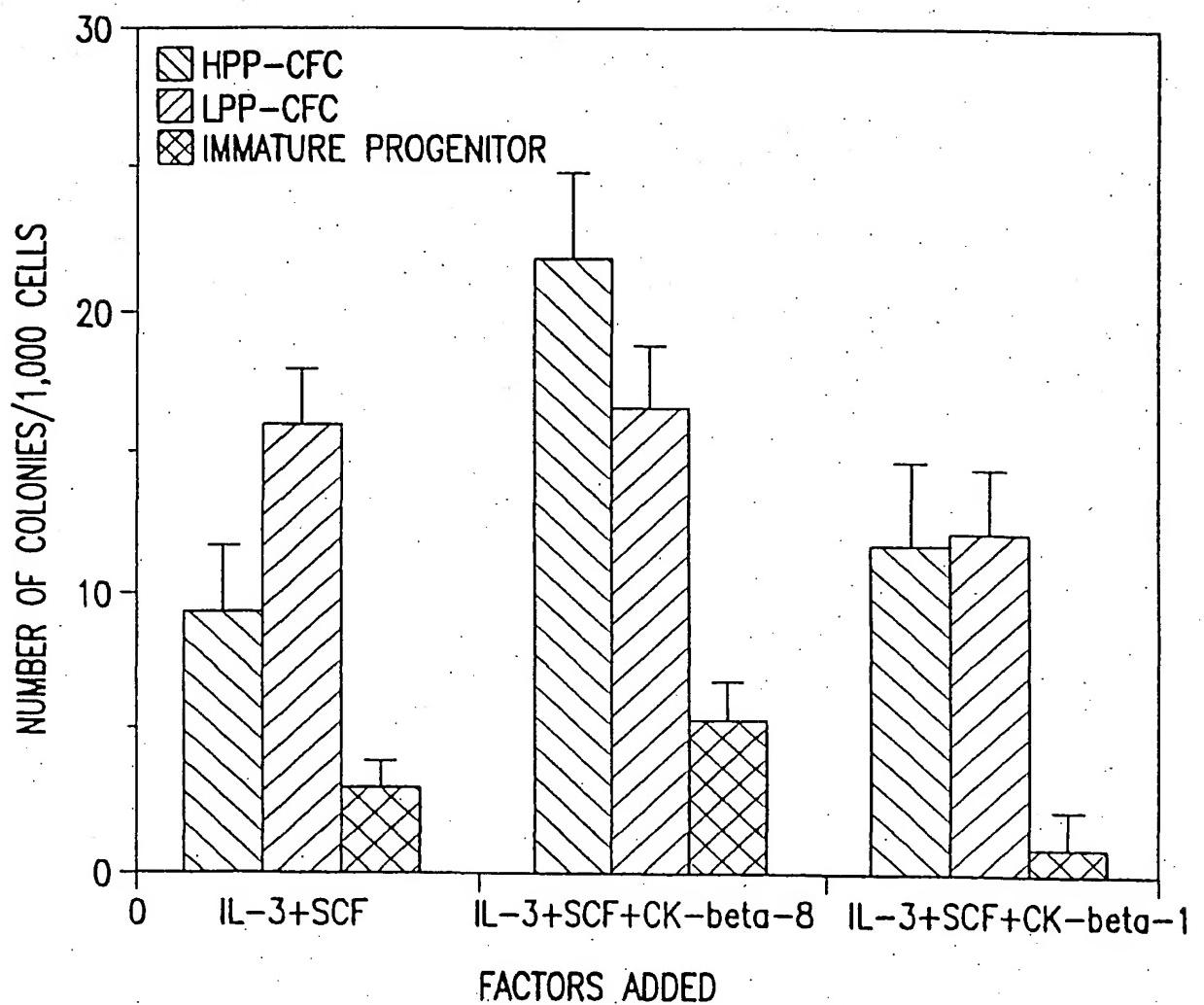


FIG.15

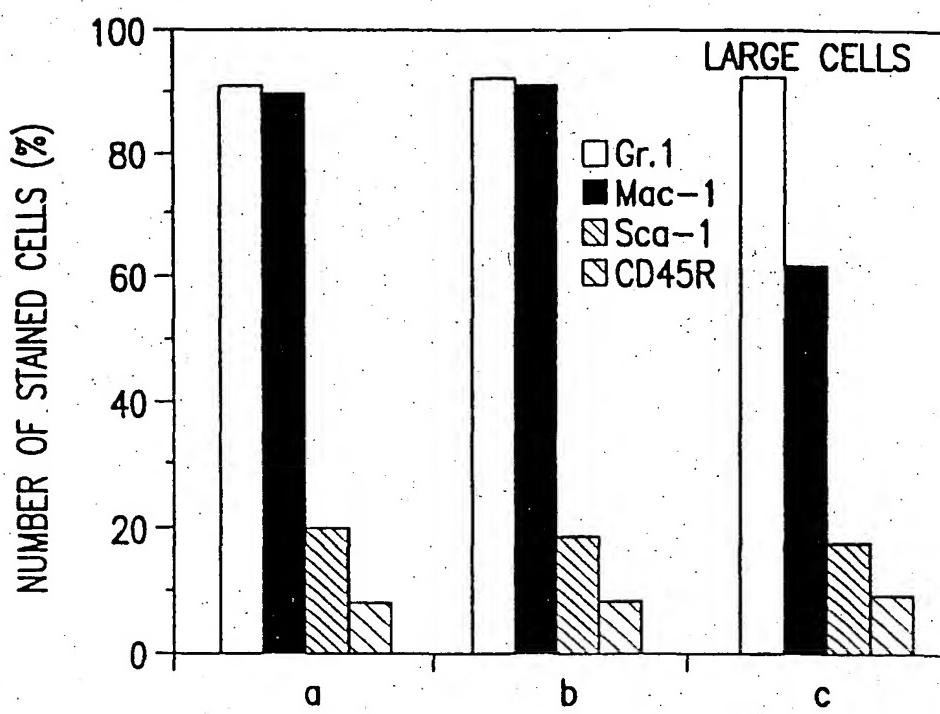


FIG.16A

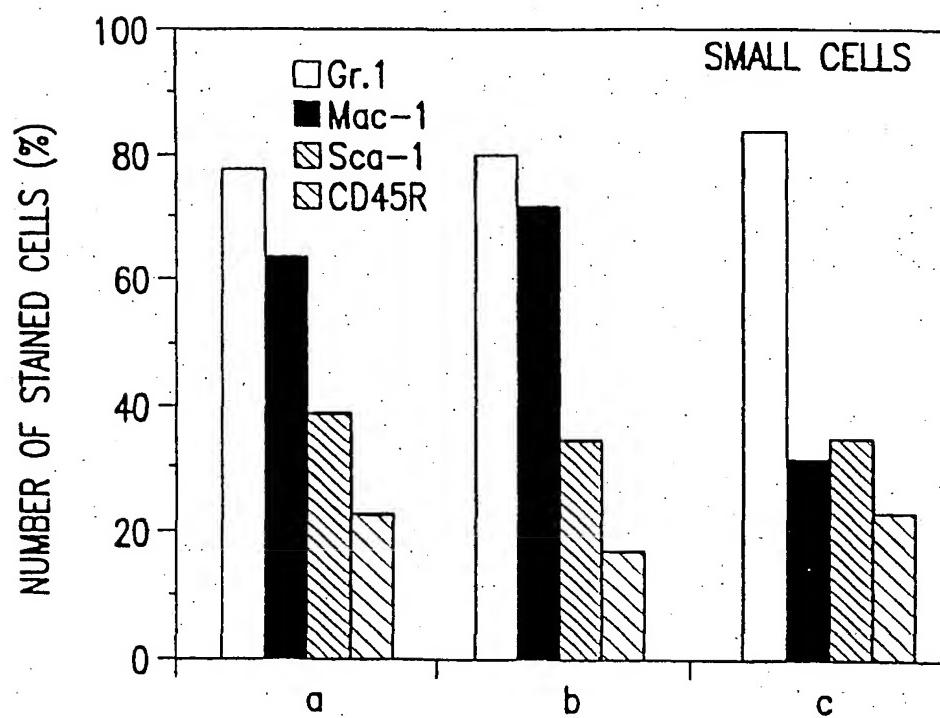


FIG.16B

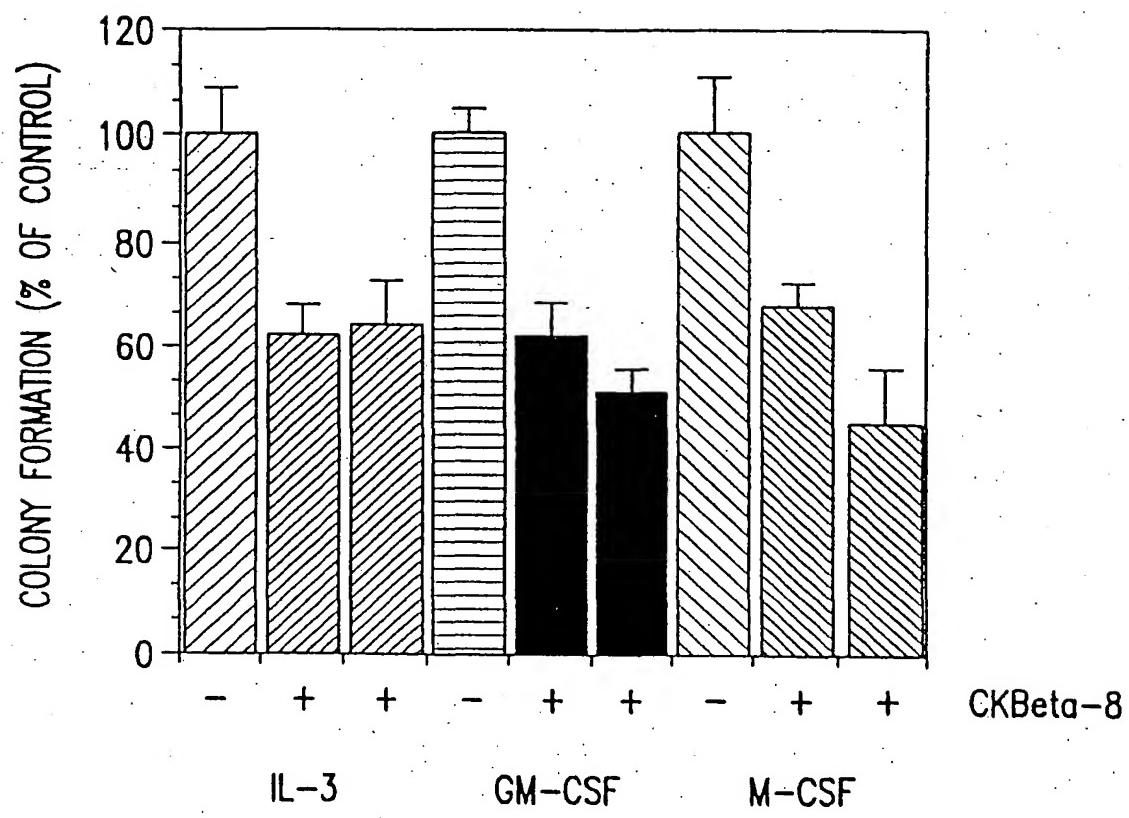


FIG.17

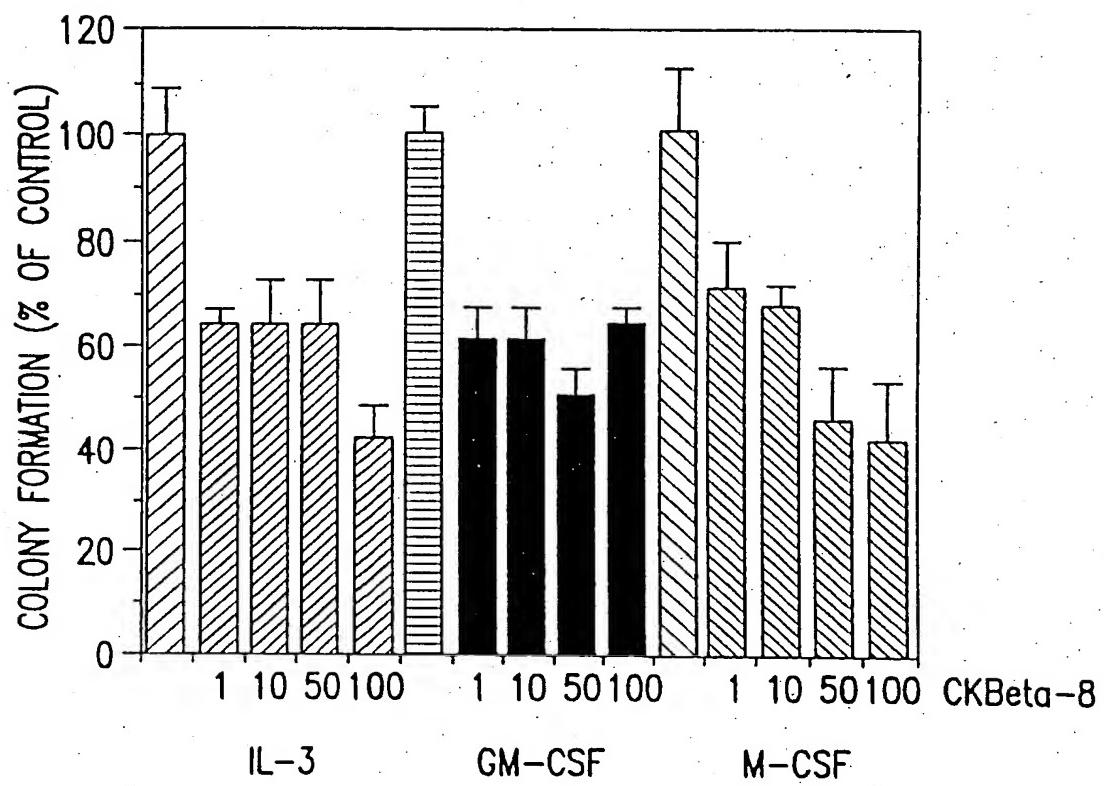


FIG.18

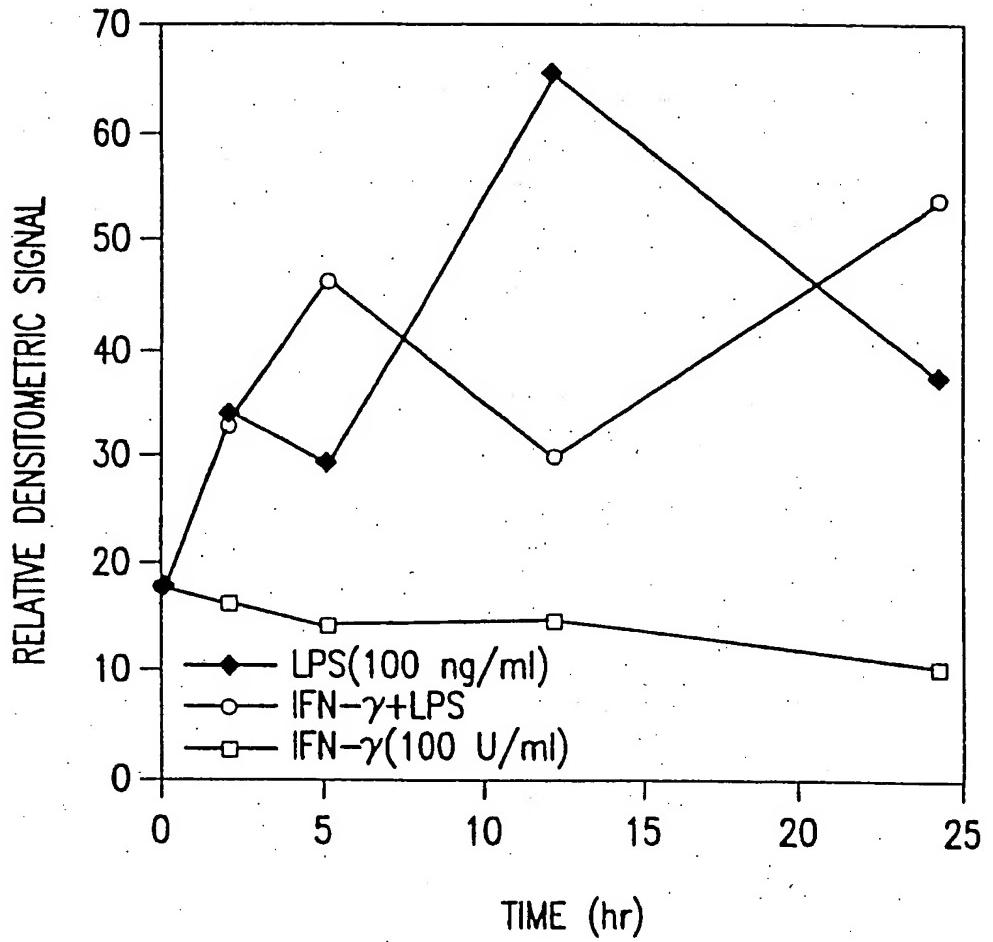


FIG.19

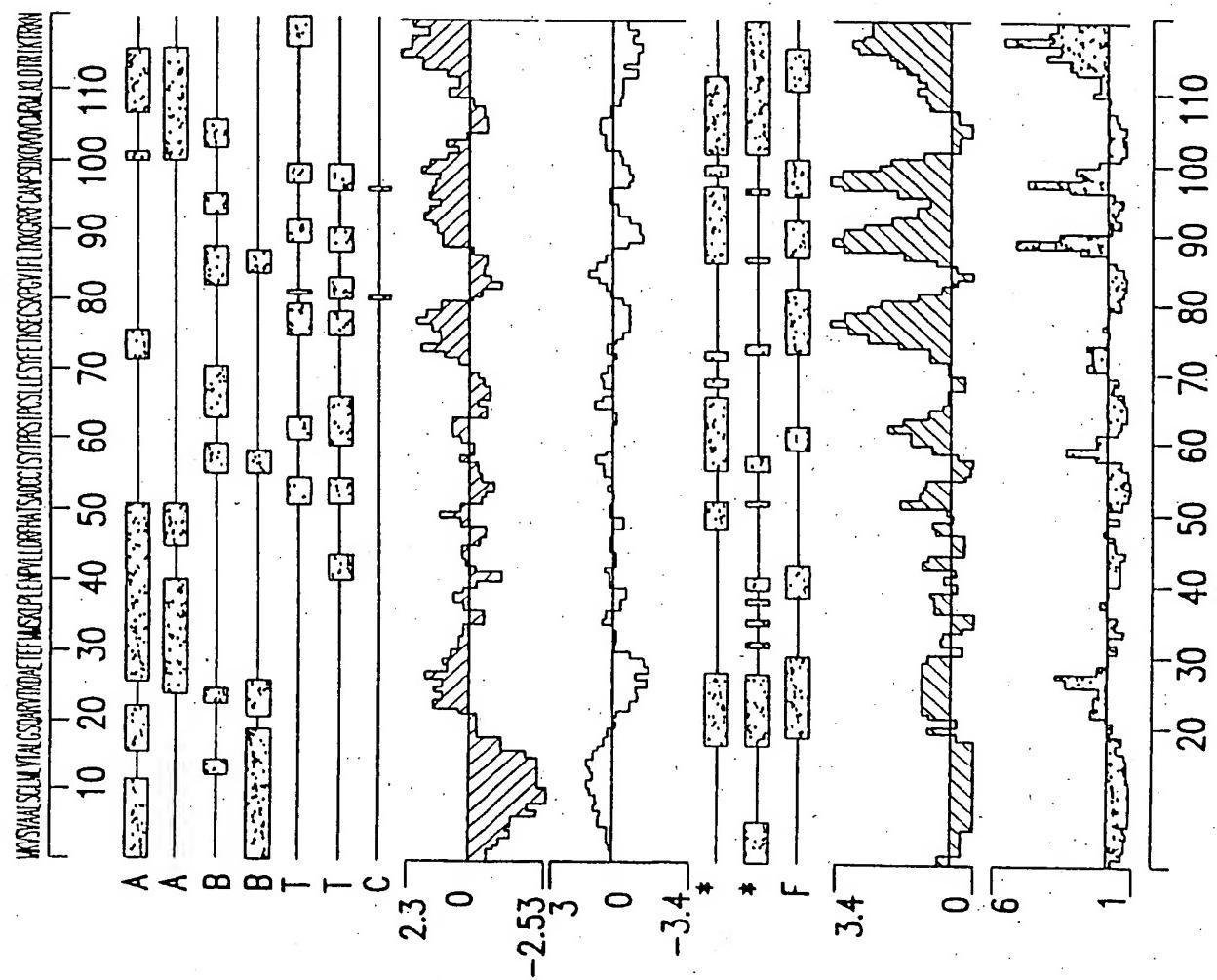
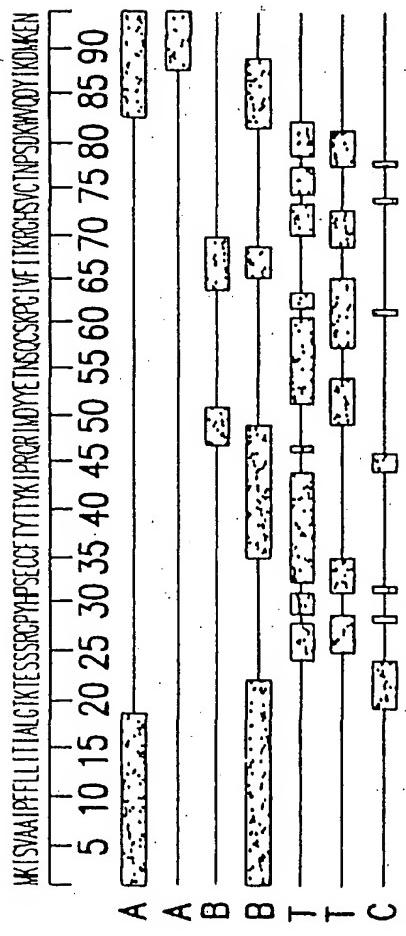


FIG. 20A

## □ SURFACE PROBABILITY PLOT-EMINI



- ALPHA, REGIONS-GARNIER-ROBSON
- ALPHA, REGIONS-CHOU-FASMAN
- BETA, REGIONS-GARNIER-ROBSON
- BETA, REGIONS-CHOU-FASMAN
- TURN, REGIONS-GARNIER-ROBSON
- TURN, REGIONS-CHOU-FASMAN
- COIL, REGIONS-GARNIER-ROBSON

□ HYDROPHILICITY PLOT-KYTE-DOOLITTLE

□ HYDROPHOBICITY PLOT-HOPP-WOODS

- ALPHA, AMPHIPATHIC REGIONS-EISENBERG
- BETA, AMPHIPATHIC REGIONS-EISENBERG
- FLEXIBLE REGIONS-KARPLUS-SCHULZ

□ ANTIGENIC INDEX-JAMESON-WOLF

□ SURFACE PROBABILITY PLOT-EMINI

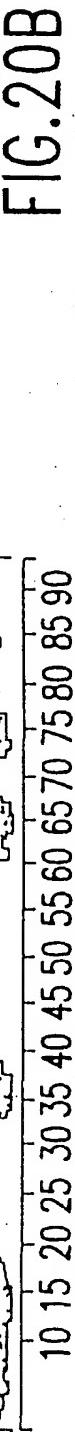


FIG. 20B

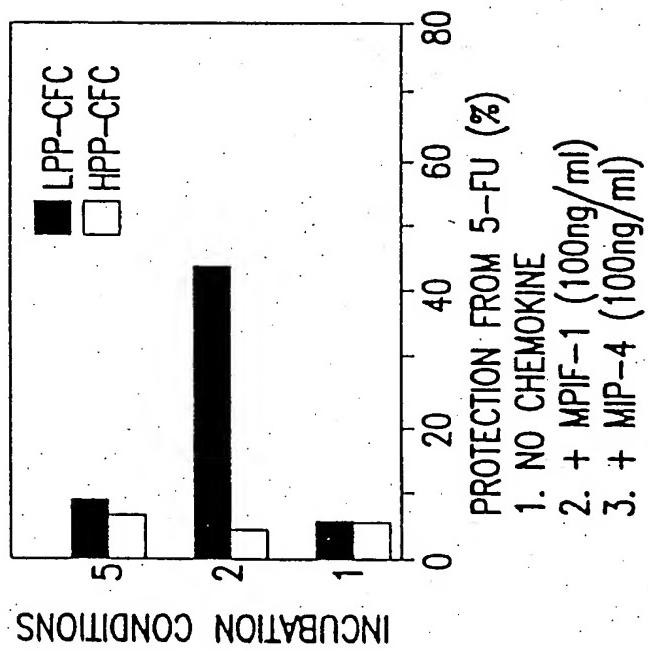
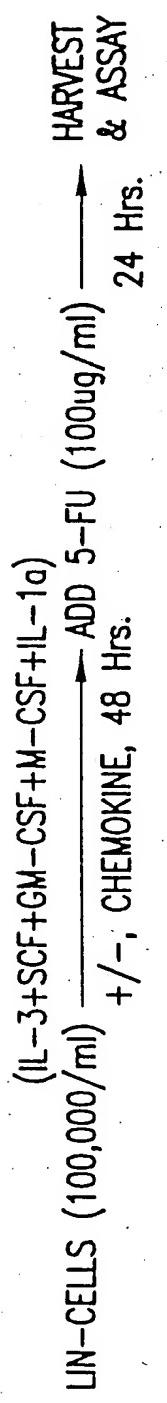
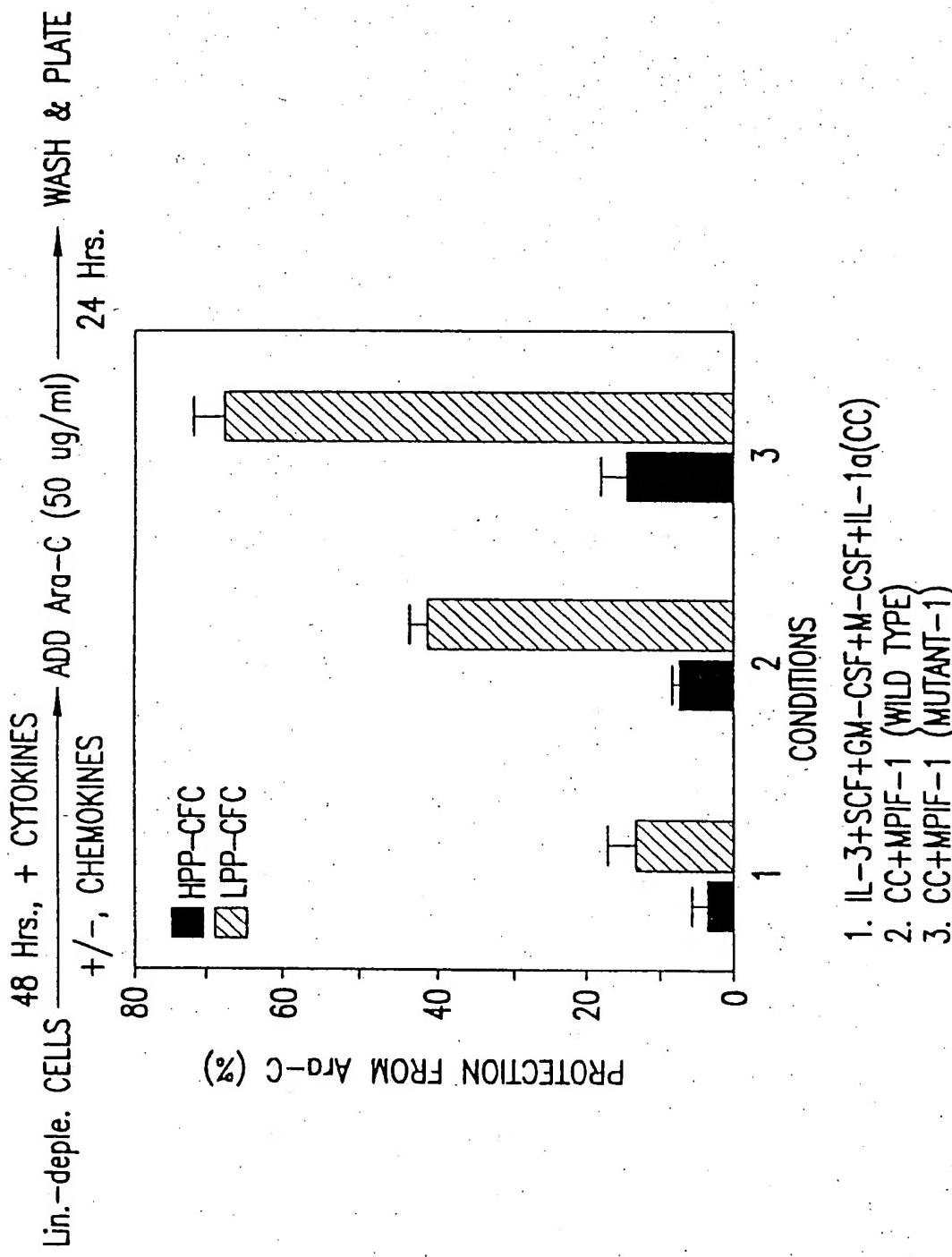


FIG.21A



**FIG. 21B**

TREATMENTS	NUMBERS OF CIRCULATING WBC PER MILLILITER OF BLOOD		
	DAY 3	DAY 6	DAY 10
Gr-1 (Saline)	$8.4 \times 10^6 \pm 3.0 \times 10^6$	$10.2 \times 10^6 \pm 3.6 \times 10^6$	$7.0 \times 10^6 \pm 9.9 \times 10^6$
Gr-2, MPIF-1 ALONE	$7.8 \times 10^6 \pm 2.2 \times 10^6$ (100%)	$7.5 \times 10^6 \pm 6.5 \times 10^5$ (100%)	$10.6 \times 10^6$ (100%)
Gr-3, 5-Fu ALONE	$4.23 \times 10^6 \pm 2.8 \times 10^6$ (54)	$1.8 \times 10^6 \pm 1.4 \times 10^4$ (24)	$8.8 \times 10^6 \pm 4.9 \times 10^5$ (83)
Gr-4, MPIF-1 PLUS 5-Fu	$3.49 \times 10^6 \pm 6.5 \times 10^5$ (45)	$3.98 \times 10^6 \pm 4.3 \times 10^5$ (53)	$9.48 \times 10^6 \pm 9.4 \times 10^5$ (89)

FIG. 22

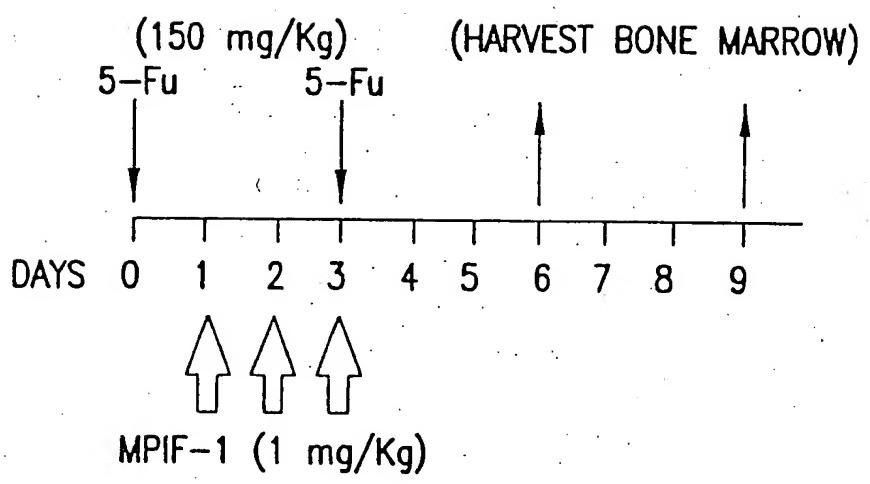


FIG.23

GROUP	TREATMENTS	NUMBER OF COLONIES PER 2,000 CELLS			
		DAY 6		DAY 9	
		HPP-CFC	LPP-CFC	HPP-CFC	LPP-CFC
1	SALINE	10.5 ± 0.7	60 ± 9.8	15 ± 2	78 ± 3.5
	SALINE	12 ± 0.7	92 ± 11	13 ± 1	80 ± 14
	SALINE	14 ± 1.4	84 ± 1.4	11 ± 2	82 ± 0
2	5-Fu	4.5 ± 3.5	3.5 ± 0.7	7 ± 2	5 ± 0
	5-Fu	12 ± 2	37 ± 16	6 ± 2	2 ± 0
	5-Fu	4 ± 2.8	6 ± 3	DEAD	DEAD
	5-Fu PLUS MPIF-1	0	6.5 ± 3.5	16 ± 1.4	75 ± 1.4
3	" " "	0	105 ± 10	12 ± 2.8	46 ± 12
	" " "	0	120 ± 1.4	16 ± 0	95 ± 2.8

FIG. 24

1 MKVSVAAALSC LMLVTALGSQ ARVTKDAETE FMMISKLPLEN PVLLDRFHAT SADCCISYTP RSIPCSLLES YFETTNSECSK  
 10 20 30 40 50 60 70 80  
 2) Wild type: RVTKDAE.....  
 3) Mutant-1(+1): MRVTKDAE..... RFHAT ..  
 4) Mutant-2(-δ 24): DRFHAT ..  
 5) Mutant-3(-δ 23): HAT SAD ..  
 6) Mutant-5(-δ 27): AT SAD ..  
 7) Mutant-6(-δ 24): MRFHAT ..  
 8) Mutant-7(-δ 17): EN PVLLD ..  
 9) Mutant-8(-δ 22): LDRFHAT ..  
 10) Mutant-9(-δ 25): HAAGFHAT ..

FIG. 25

gtcctcgccagccctgcctgcccaccaggaggatgaaggtctccgtggctgcccttcgcctcatgctt  
M K V S V A A L S C L M I  
 ttactgcccttgatcccaggccccgggtcacaaaagatgcagagacagagttcatgtgtcaaagcttcca  
V T A L G S O A R V T K D A E T E F M M S K L P  
 ttggaaaatccagtacttctggacatgctctggaggagaagattggcctcagatgacccttctcatgcc  
L E N P V L L D M L W R R K I G P Q M T L S H A  
 gcaggattccatgctacttagtgactgctgcatctcctacaccccaacgaagcatccgtgttcactcctg  
A G F H A T S A D C C I S Y T P R S I P C S L L  
 gagagttactttgaaacgaacagcgagtgctccaagccgggtgtcatttcctcaccagaagaaggggcgacgt  
E S Y F E T N S E C S K P G V I F L T K K G R R  
 ttctgtgccaacccagtgataaggcaagttcaggtttgcatgagaatgctgaagctggacacacggatcaag  
F C A N P S D K Q V Q V C M R M L K L D T R I K  
 accaggaagaattgaacttgtcaaggtaagggacacaagttgccagccaccaactttctgcctcaactaa  
T R K N \*  
 cttcctgaatttttttaagaagcatttattctgtgttggatttagagcaattcatcttcacc  
 tttaaaaaaaaaaaaaaaaaaaa

## FIG.26A

1	MKVSVAAALSCLMLVTALGSQARVTKDAETEFMM SKLPLENPVLLDMWRR	50	MPIF-1 variant
1	MKVSVAAALSCLMLVTALGSQARVTKDAETEFMM SKLPLENPVLLDR....	46	MPIF-1
51	KIGPQMTLSHAAGFHATSADCCISYTPRSIPCSLLESYFETNSECSKPGV	100	
47	.....FHATSADCCISYTPRSIPCSLLESYFETNSECSKPGV	83	
101	IFLTGKGRRFCANPSDKQVQVCMRMLKLDTRIKTRKN	137	
84	IFLTGKGRRFCANPSDKQVQVCMRMLKLDTRIKTRKN	120	

## FIG.26B

MPIF-1 MUTANTS	CONCENTRATION (ng/ml)
WILD TYPE	100
PREPARATION K0871	10
MUTANT-1	50
MUTANT-6	100
HG00300-B7	10
MUTANT-9	10

FIG.27

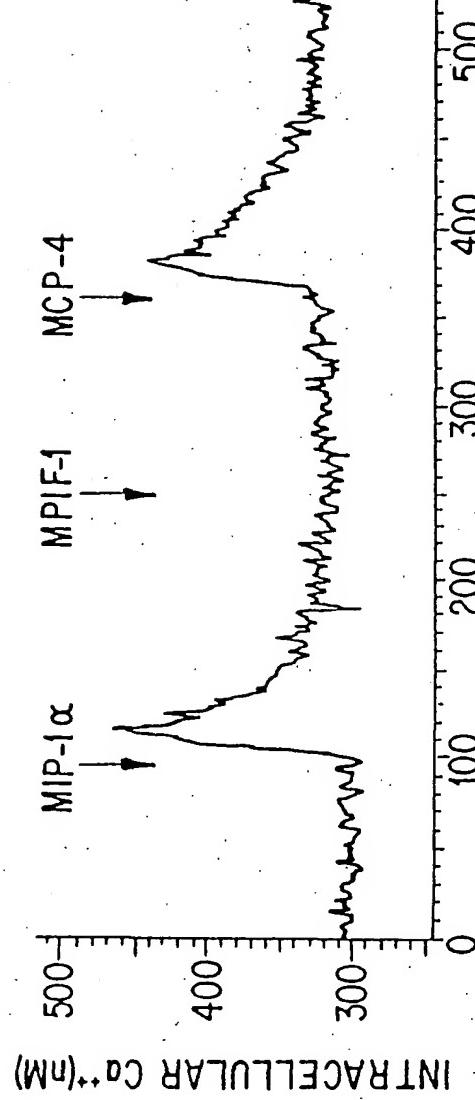


FIG. 28A

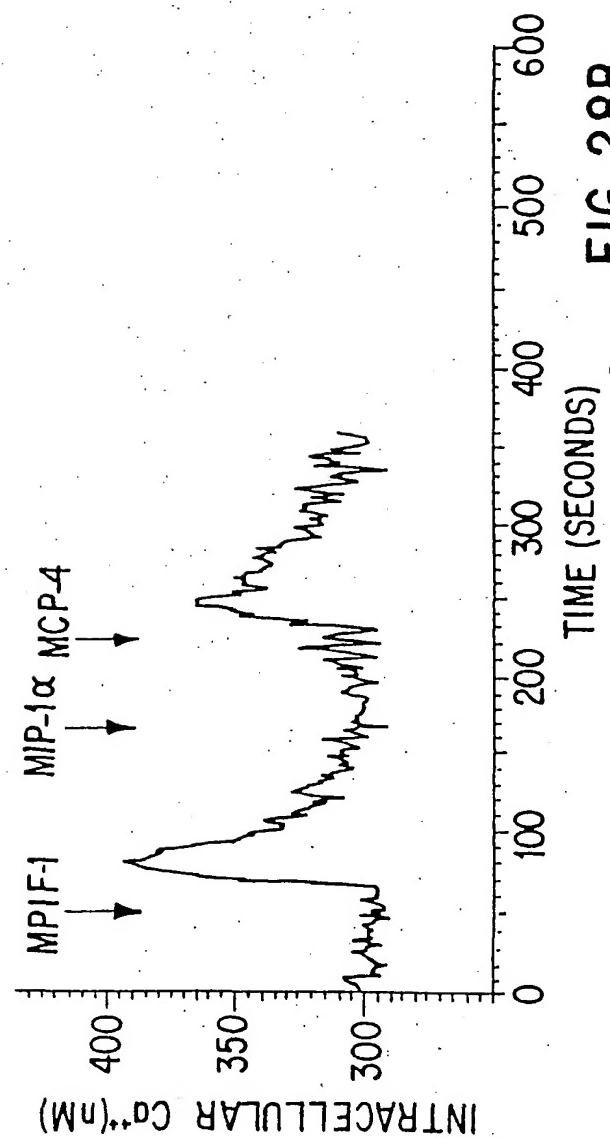


FIG. 28B

ADDITIONS	CALCIUM MOBILIZATION RESPONSE
MIP-1 $\alpha$ ALONE	+
MPIF-1 ALONE	+
MIP-1 $\alpha$ FOLLOWED BY MPIF-1	-
MPIF-1 FOLLOWED BY MIP-1 $\alpha$	-
MIP-1 $\alpha$ FOLLOWED BY:	
PREPARATION K0871	-
HG00300-B7	-
MUTANT-6	-
MUTANT-1	-
MUTANT-9	-
PREPARATION K0871	+
K0871 FOLLOWED BY MIP-1 $\alpha$	-
HG00300-B7	+
HG00300-B7 FOLLOWED BY MIP-1 $\alpha$	-
MUTANT-6	+
MUTANT-6 FOLLOWED BY MIP-1 $\alpha$	-
MUTANT-1	+
MUTANT-1 FOLLOWED BY MIP-1 $\alpha$	-
MUTANT-9	+
MUTANT-9 FOLLOWED BY MIP-1 $\alpha$	-

FIG.29

PROTEINS	CHEMOTAXIS *
WILD TYPE	50-100 ng/ml (3-4X)
PREPARATION K0871	10-30 ng/ml (6-7X)
MUTANT-1	50-100 ng/ml (3-4X)
MUTANT-6	50-100 ng/ml (5-7X)
HG00300-B7	10-30 ng/ml (4-5X)

FIG.30

ADDITIONS	CONCENTRATION REQUIRED FOR 50% OF MAXIMAL LPP-CFC INHIBITION (ng/ml)
MPIF-1, WILD TYPE	10-20
MUTANT-1	15-25
MUTANT-6	1-10
PREPARATION K0871	0.1-1.0
HG00300-B7	0.1-1.0

FIG.31

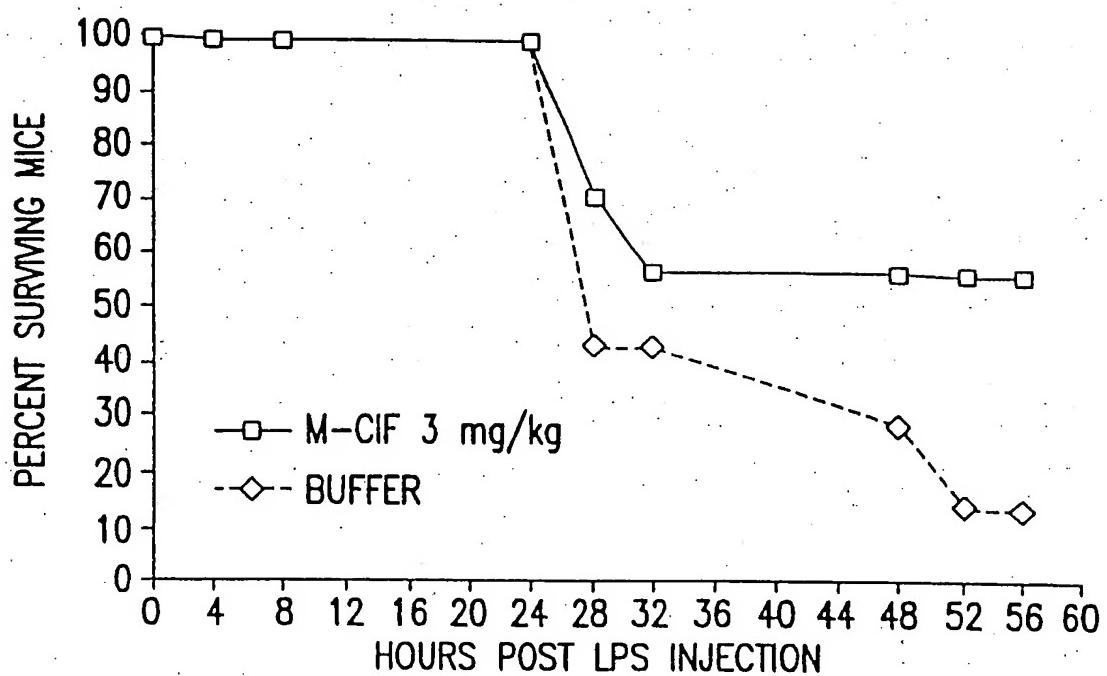


FIG.32

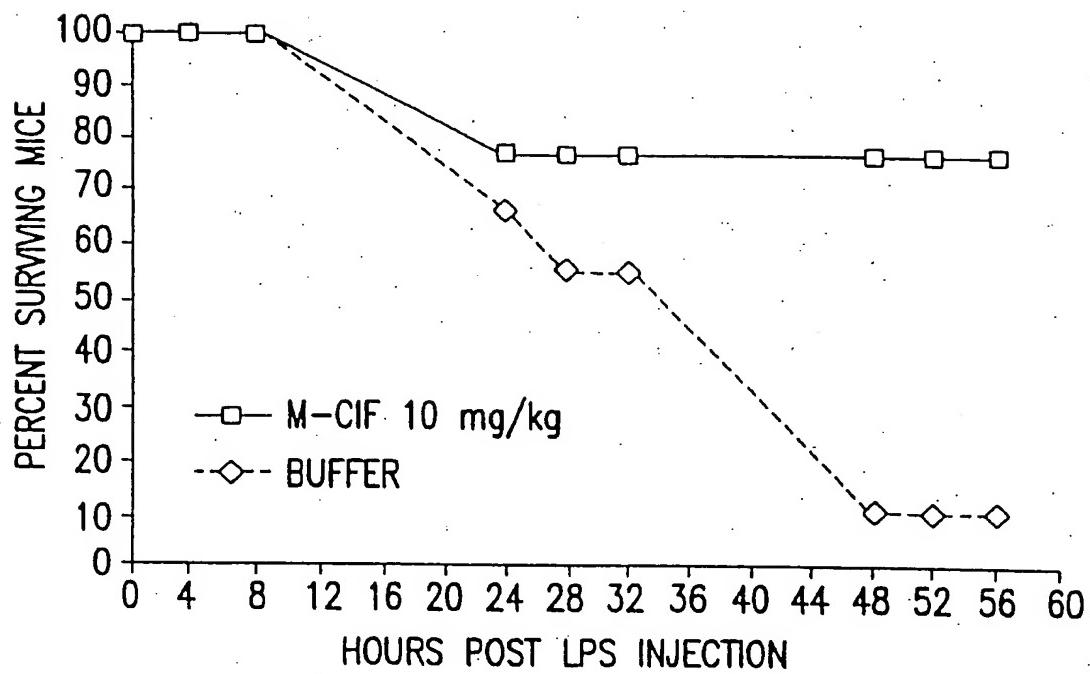


FIG.33

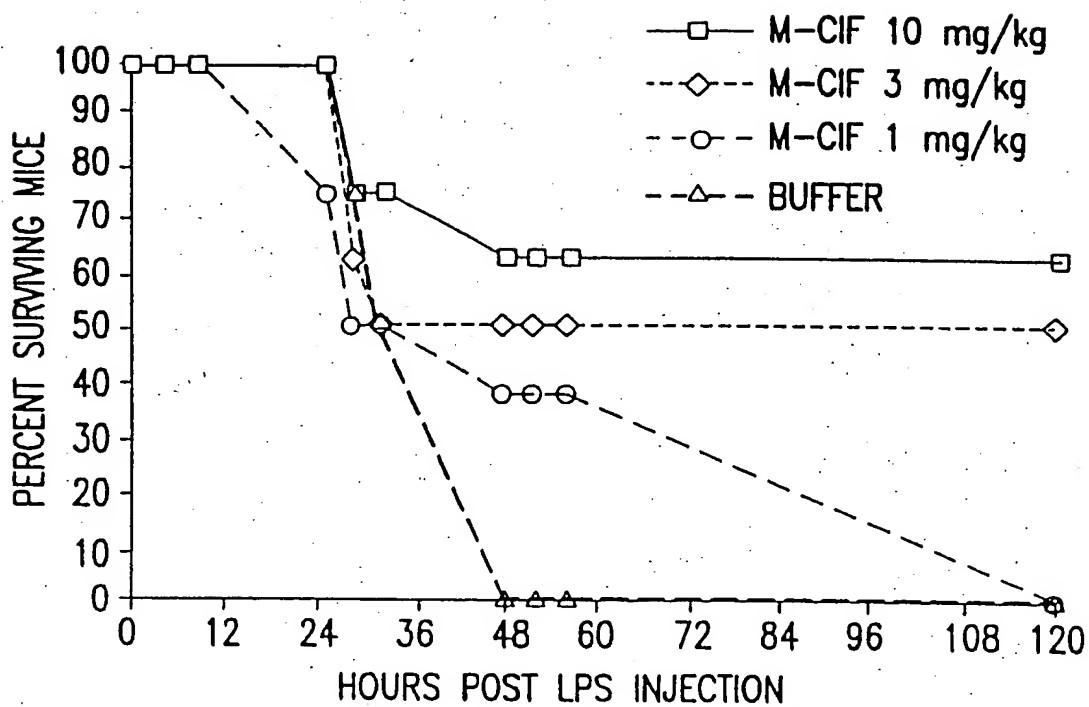


FIG.34

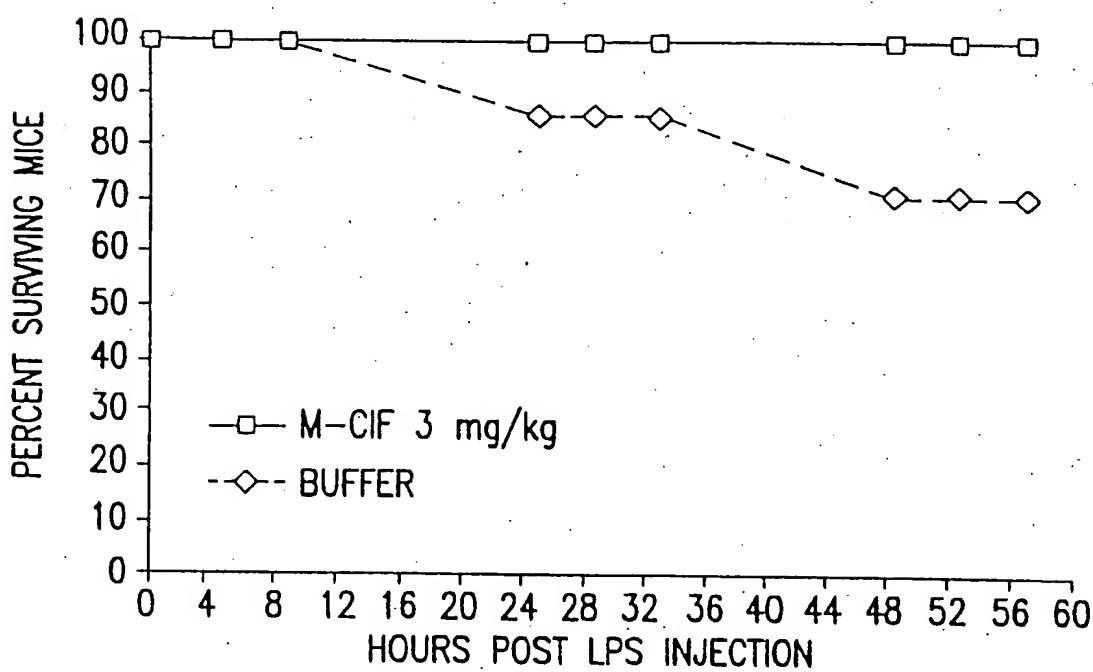


FIG.35A

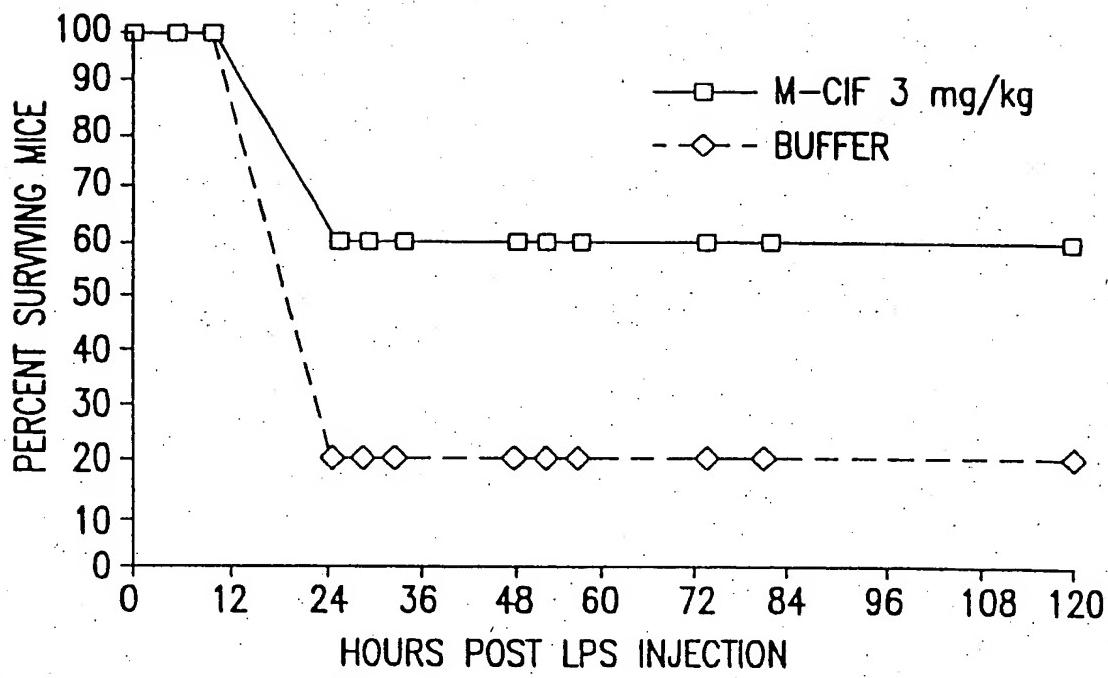


FIG.35B

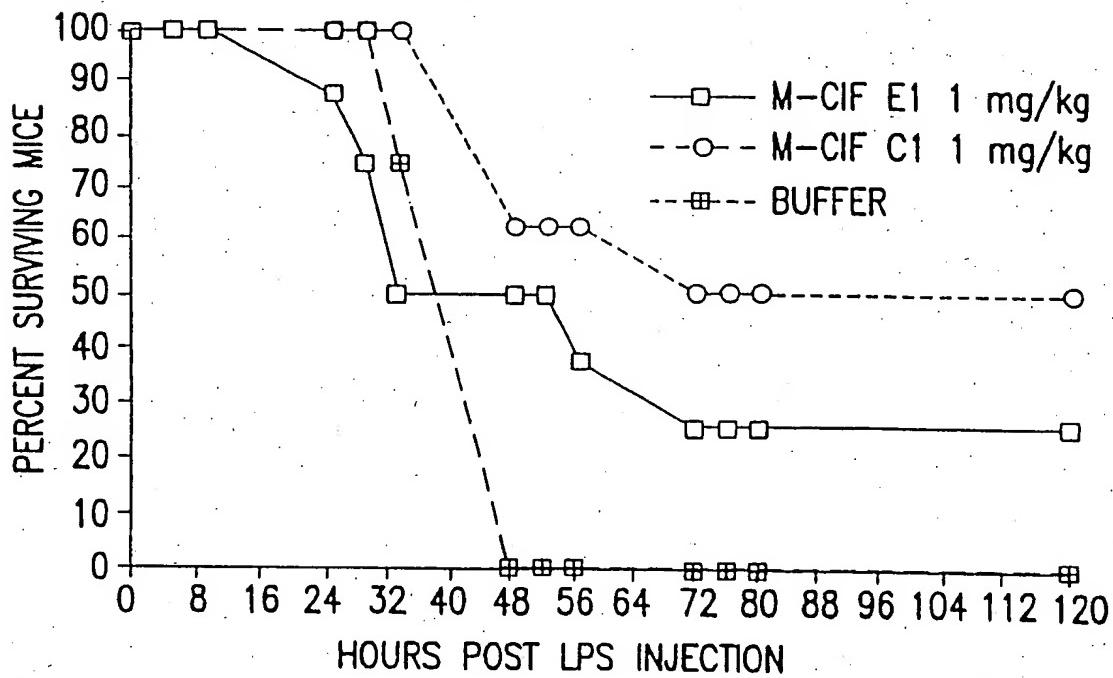


FIG.36

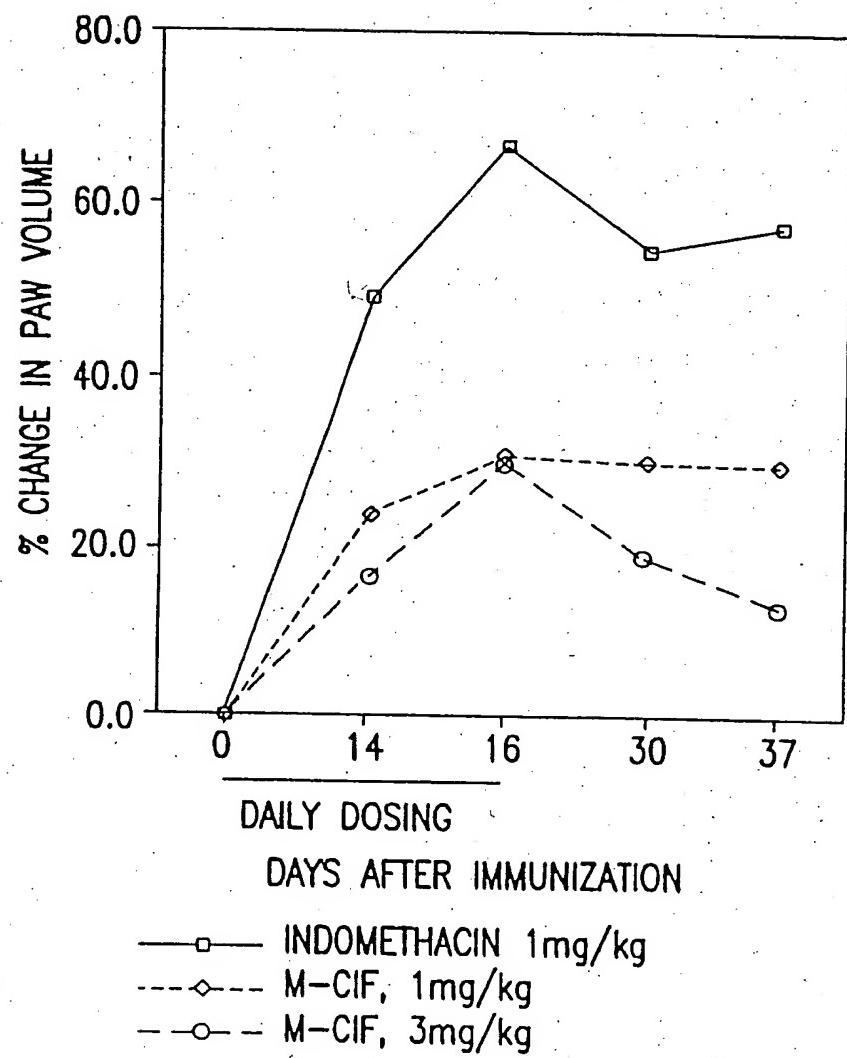


FIG.37

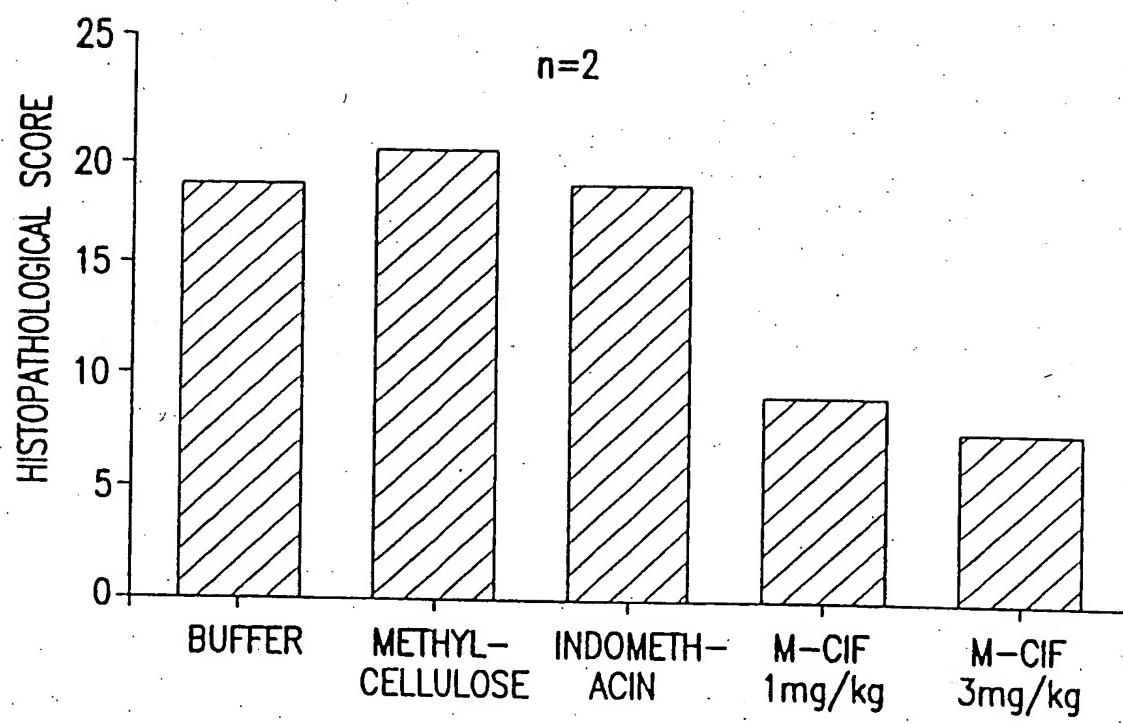


FIG.38

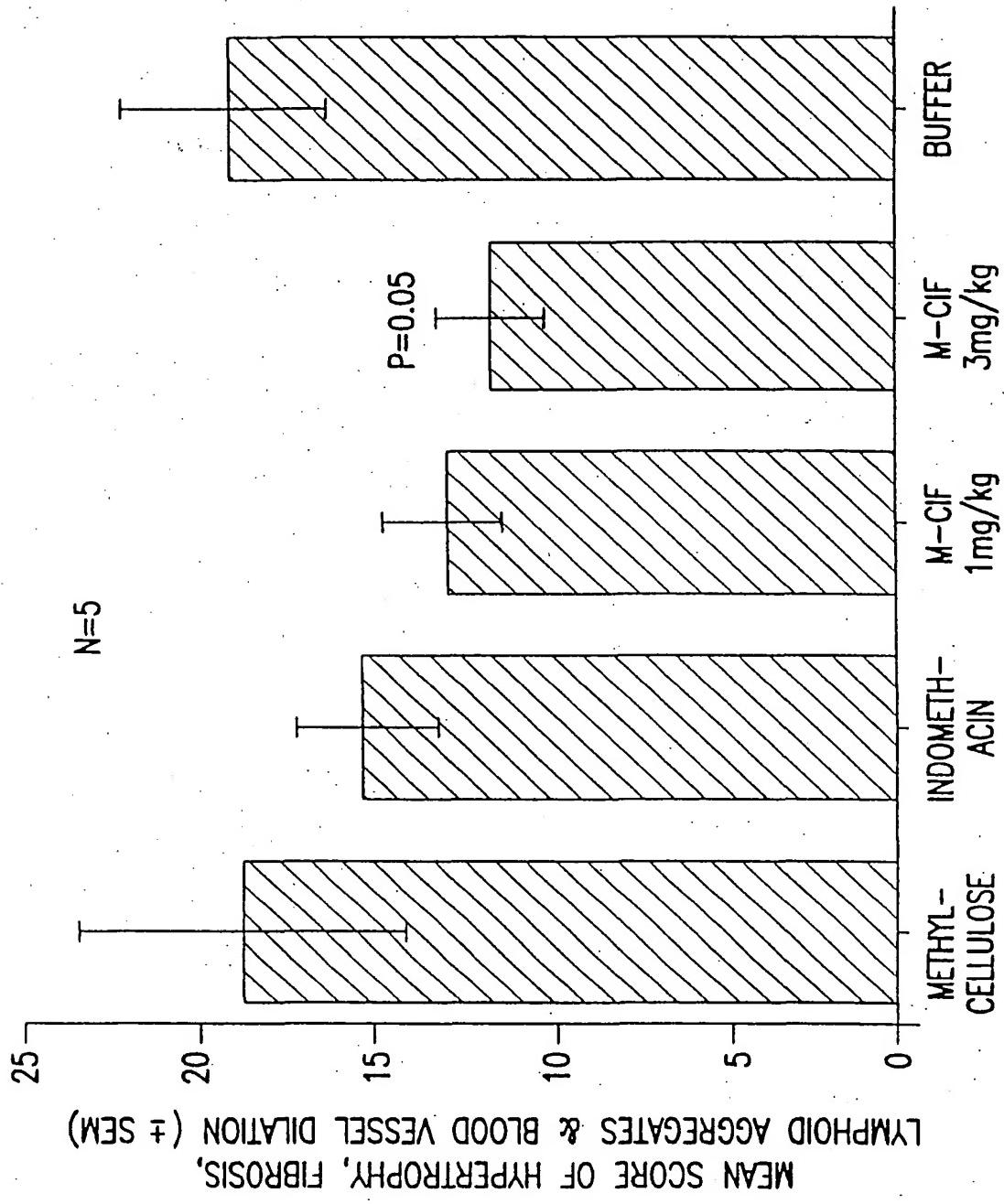


FIG. 39

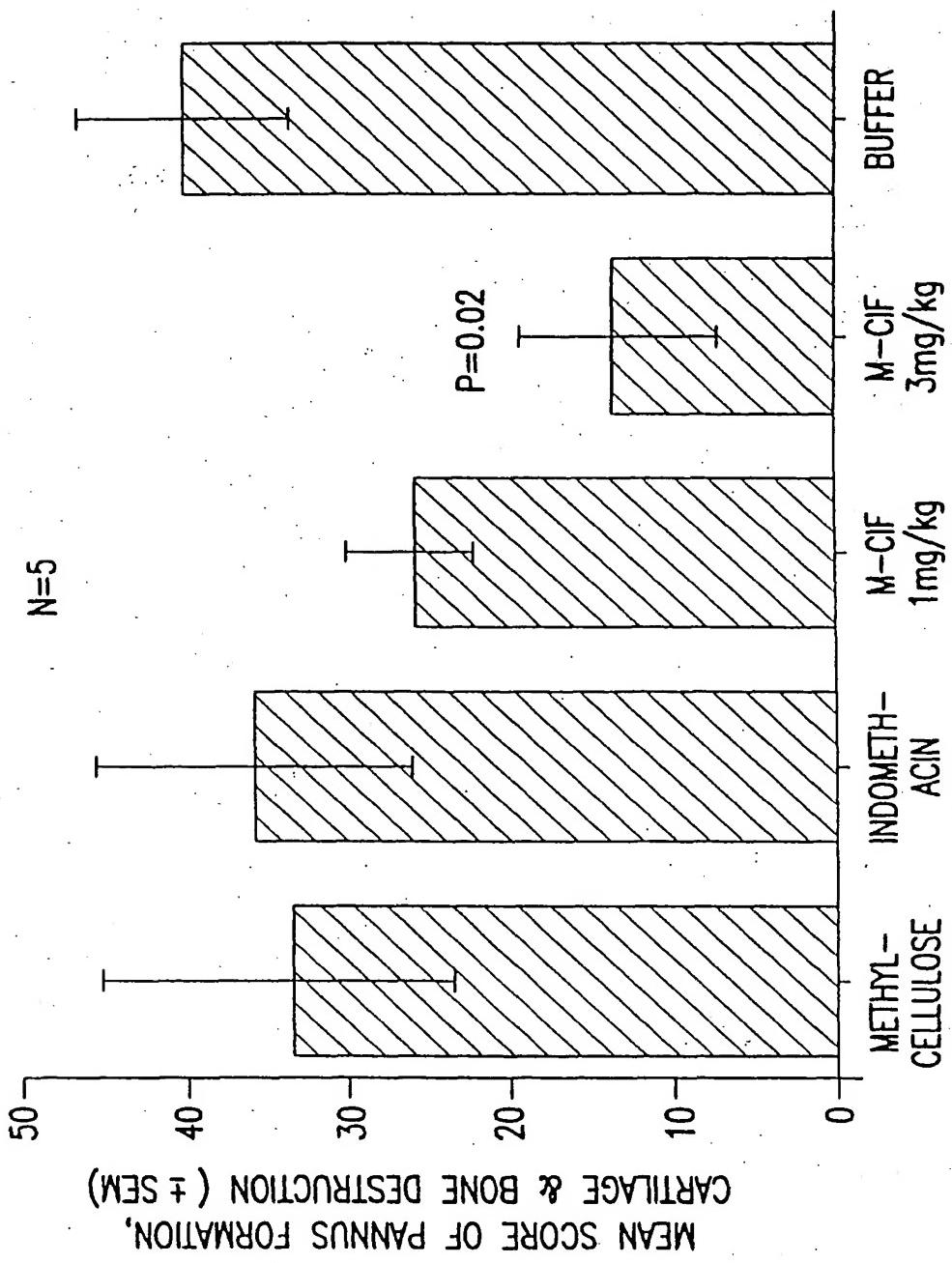


FIG. 40

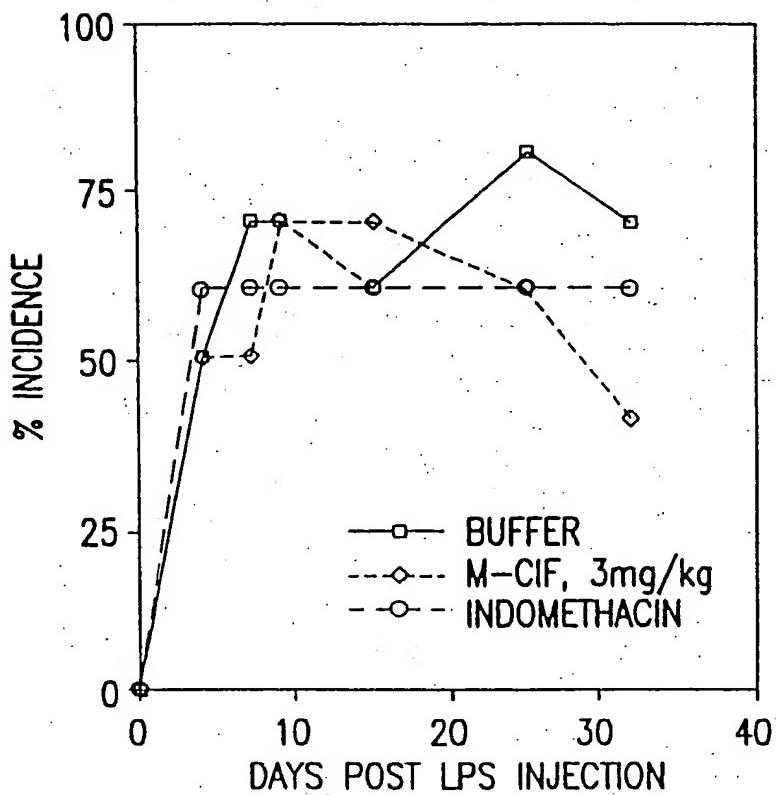


FIG. 41

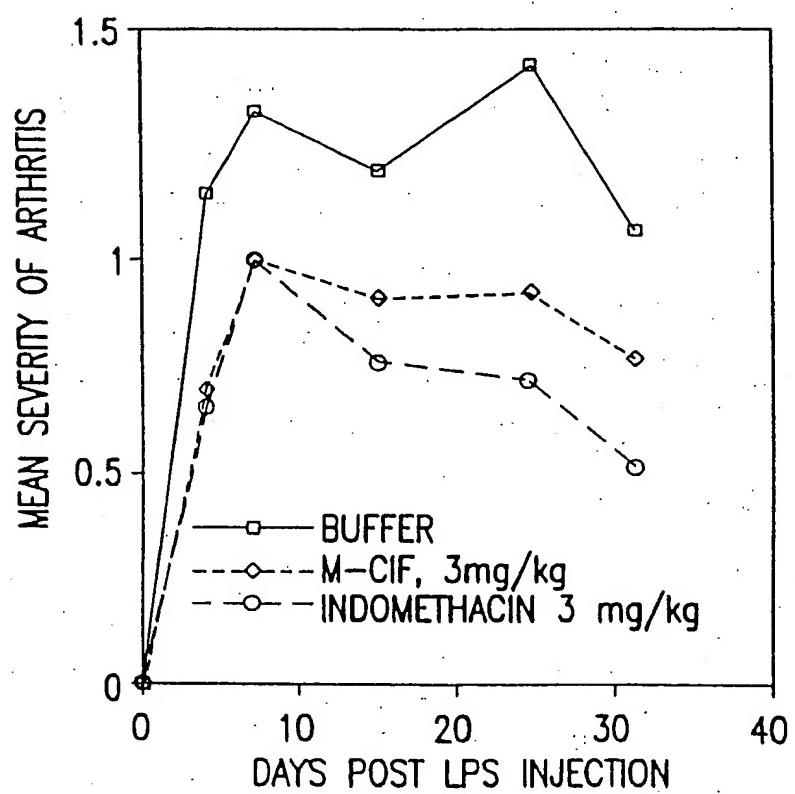


FIG. 42

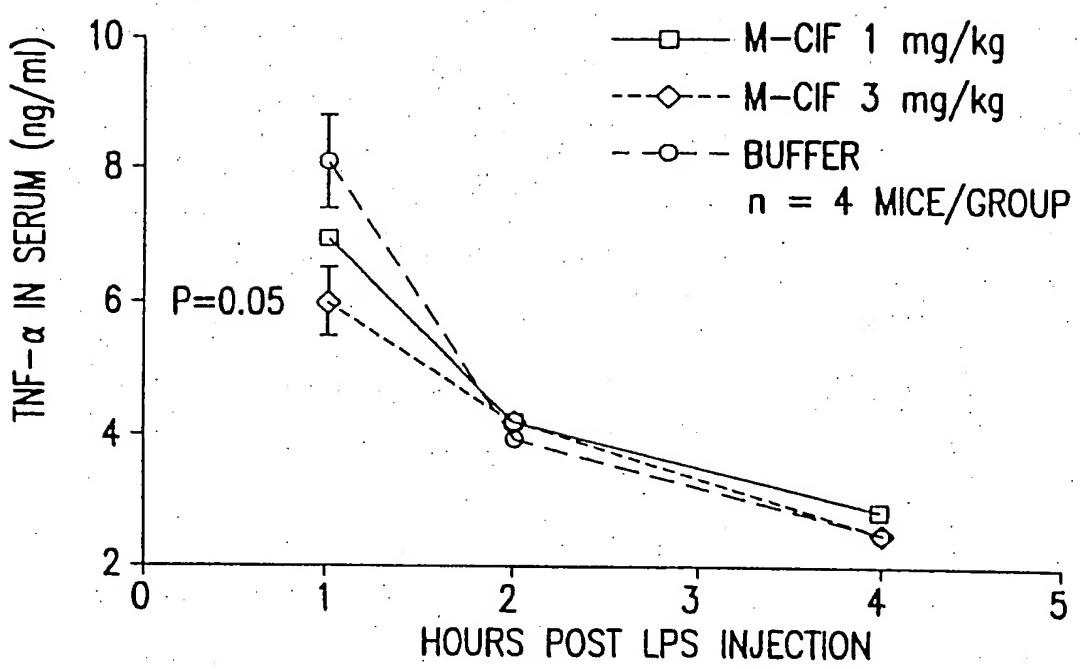


FIG.43

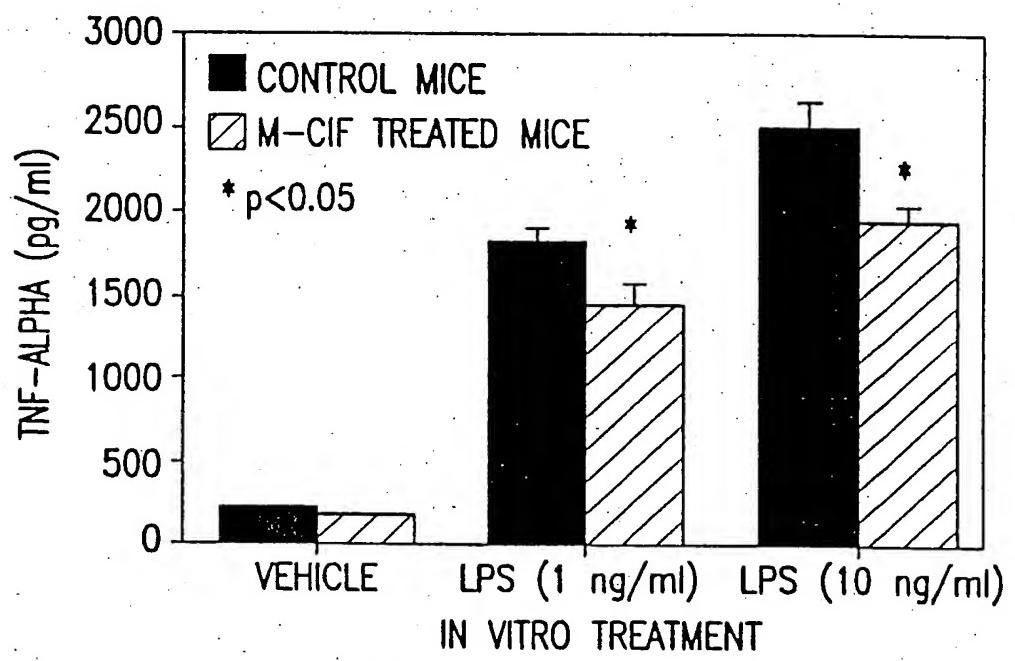
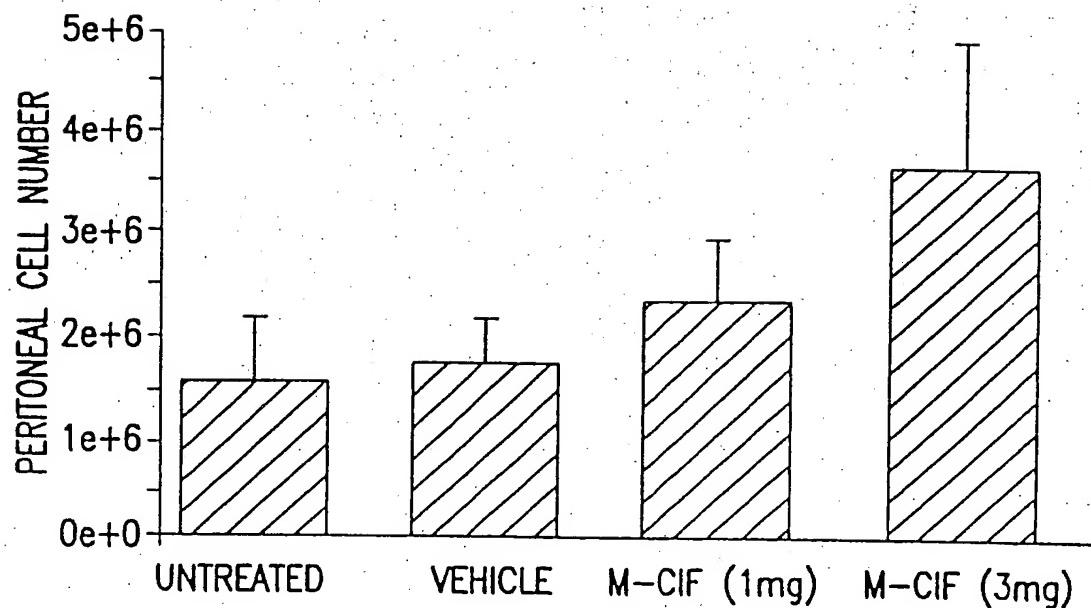


FIG.44



	UNTREATED	VEHICLE	M-CIF (1mg/kg)	M-CIF (3mg/kg)	
UNTREATED			1mg/kg		p=0.019
UNTREATED		VEHICLE	1mg/kg		p=0.027
UNTREATED				3mg/kg	p=0.0003
UNTREATED		VEHICLE		3mg/kg	p=0.0003
UNTREATED			1mg/kg	3mg/kg	p=0.007

FIG.45

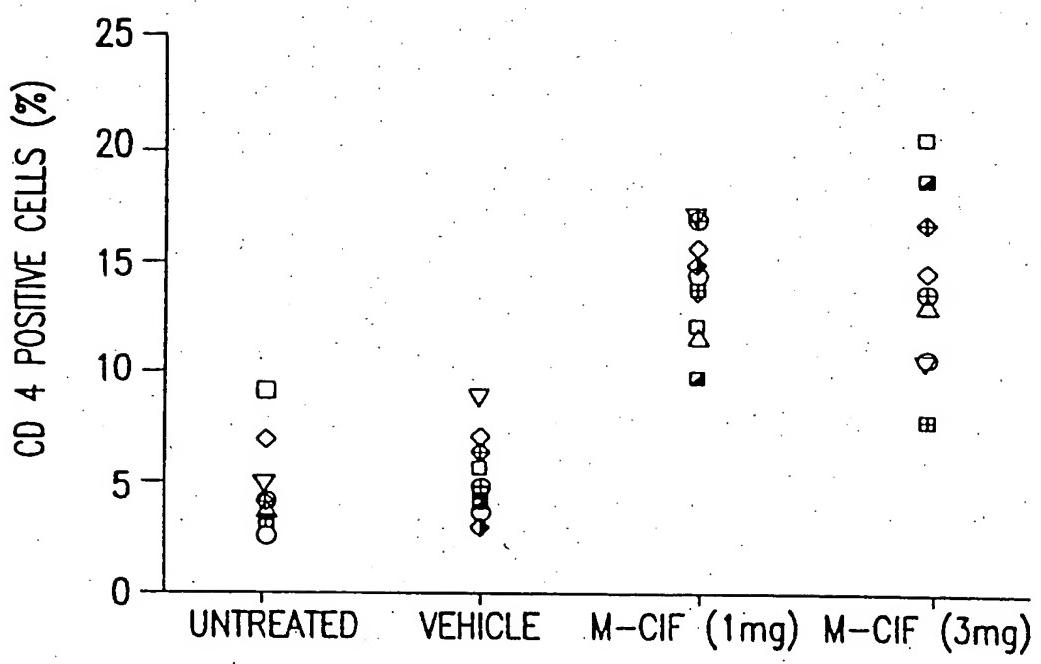


FIG.46

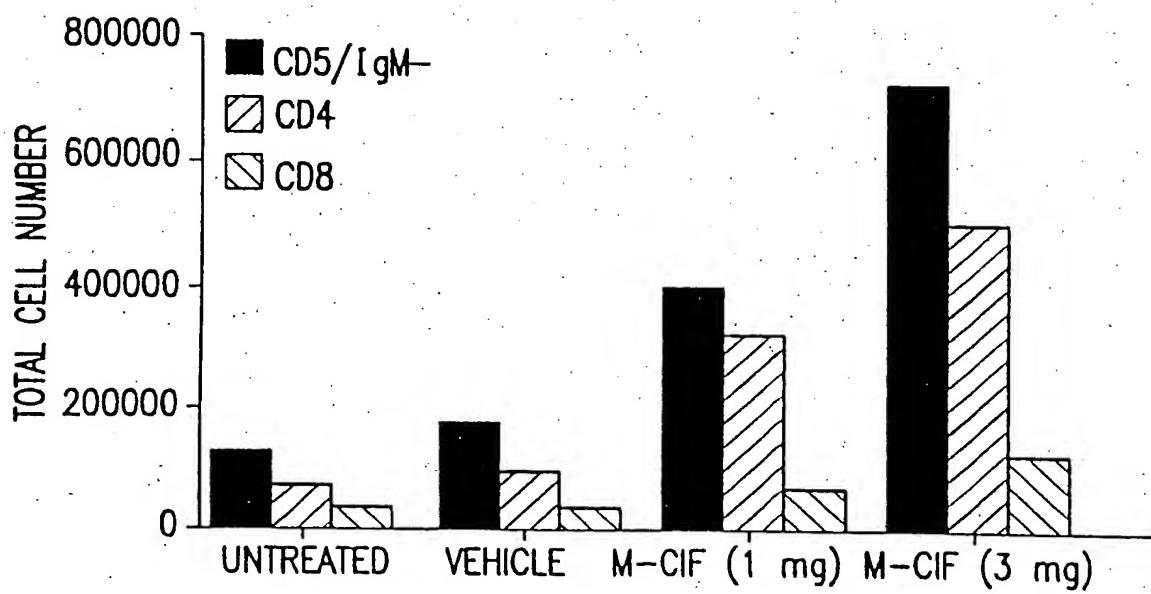


FIG.47

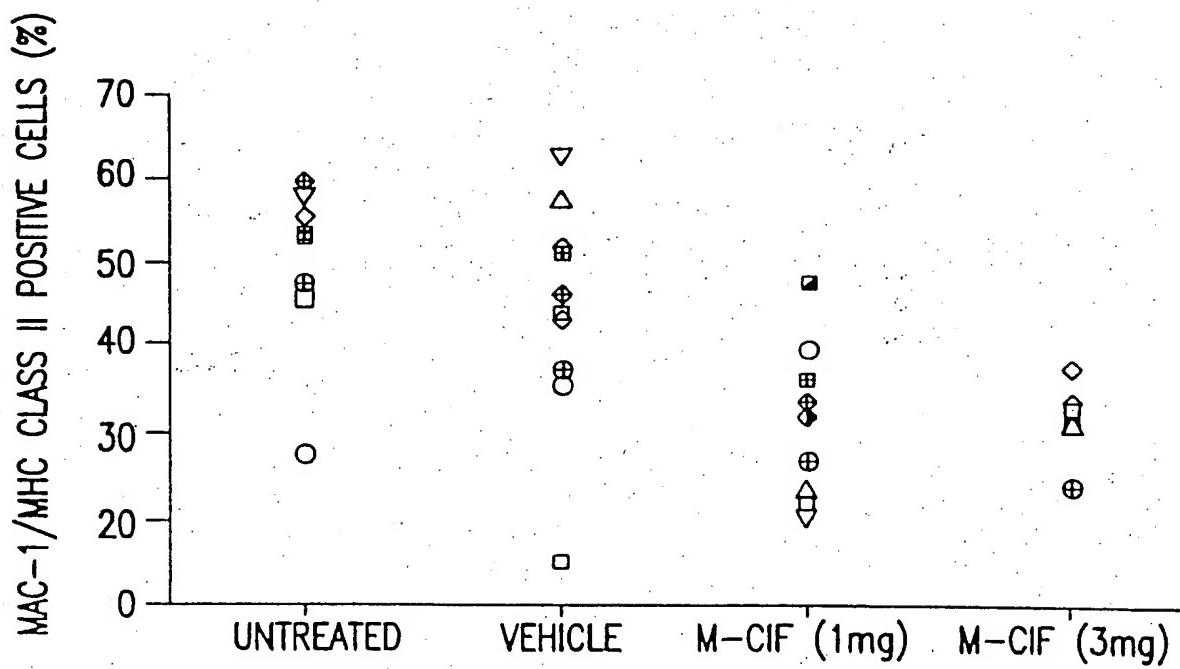


FIG.48A

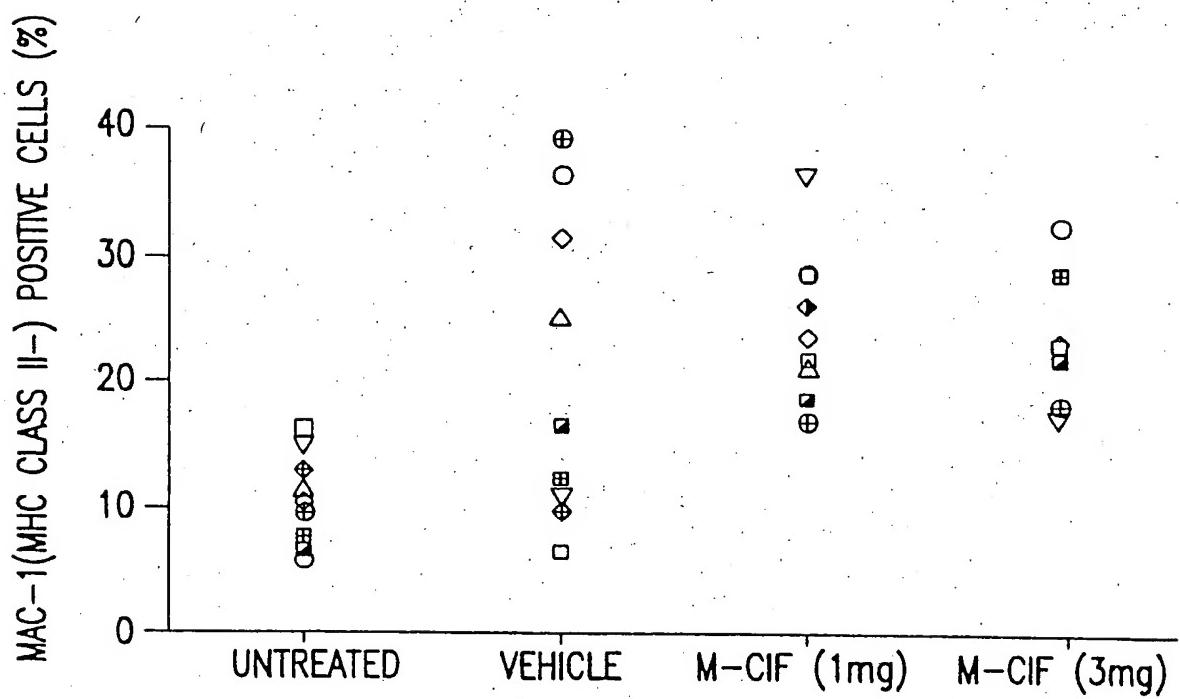


FIG.48B

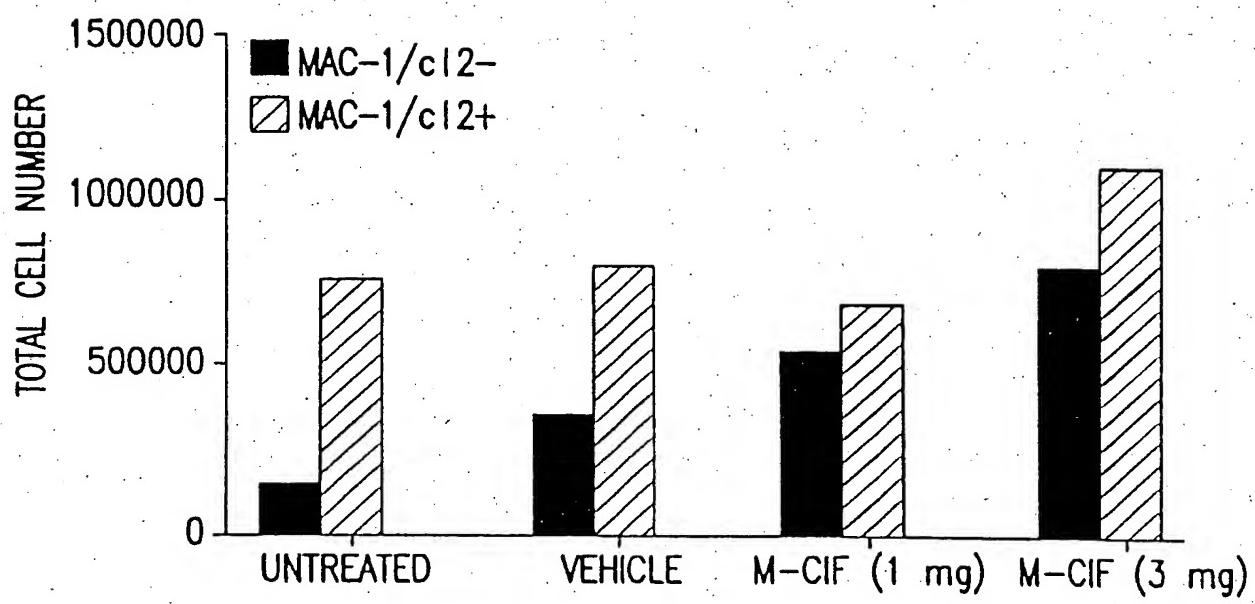


FIG.49

STEM CELL MOBILIZATION IN RESPONSE TO ADMINISTERING MPIF-1 TO NORMAL MICE			
EXPERIMENT	TREATMENTS	WBC/ml BLOOD ( $\times 10^6$ )	PHENOTYPE OF CELLS
		Gr.1	CD34 $^{+}$ Sca-1 $^{+}$
1.	SALINE MPIF-1	4.7 $\pm$ 0.36 7.1 $\pm$ 0.63	10 39
			0.20 8

FIG.50

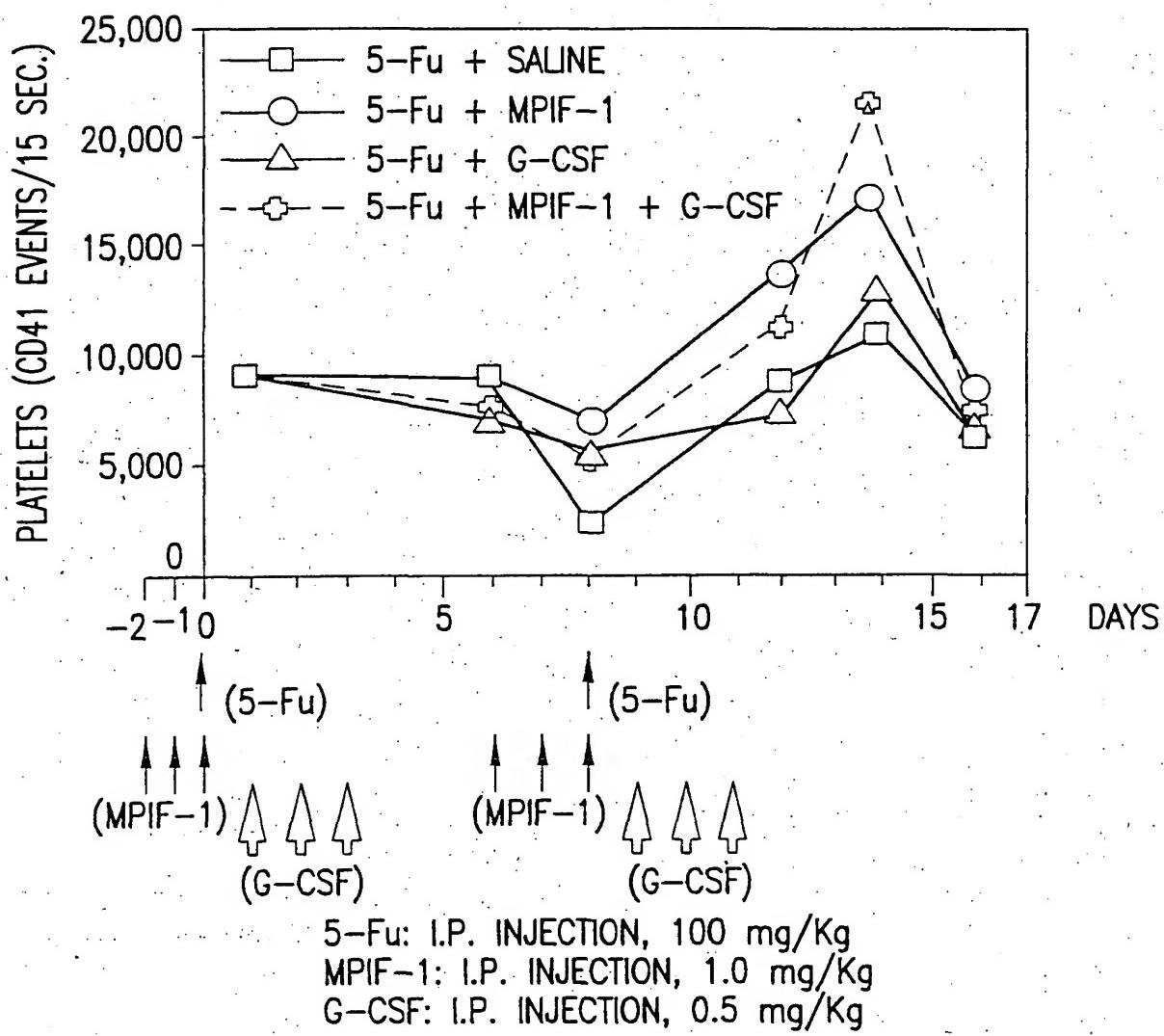
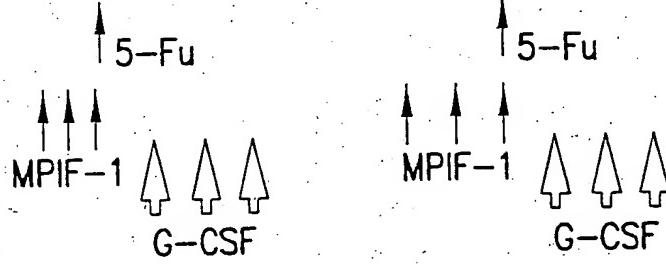
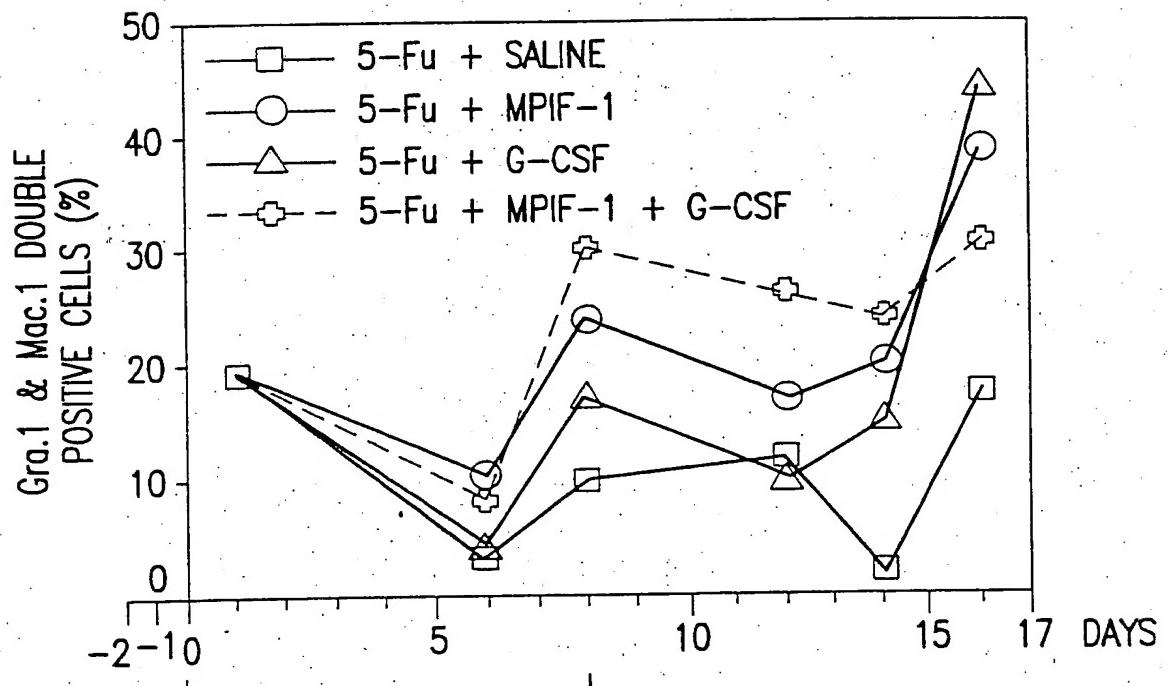


FIG.51



5-Fu: I.P. INJECTION, 100 mg/Kg  
 MPIF-1: I.P. INJECTION, 1.0 mg/Kg  
 G-CSF: I.P. INJECTION, 0.5 mg/Kg

FIG.52

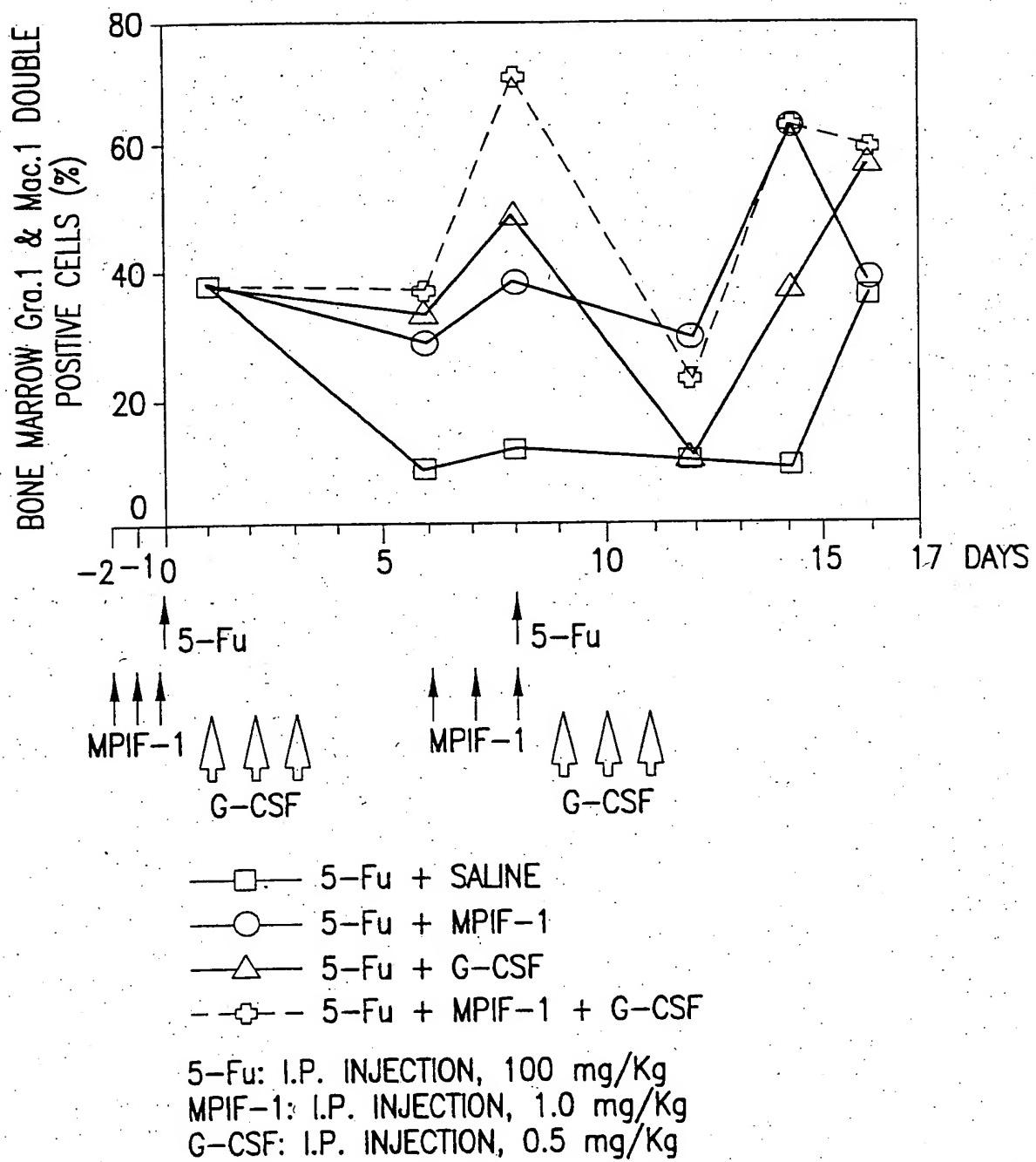


FIG.53

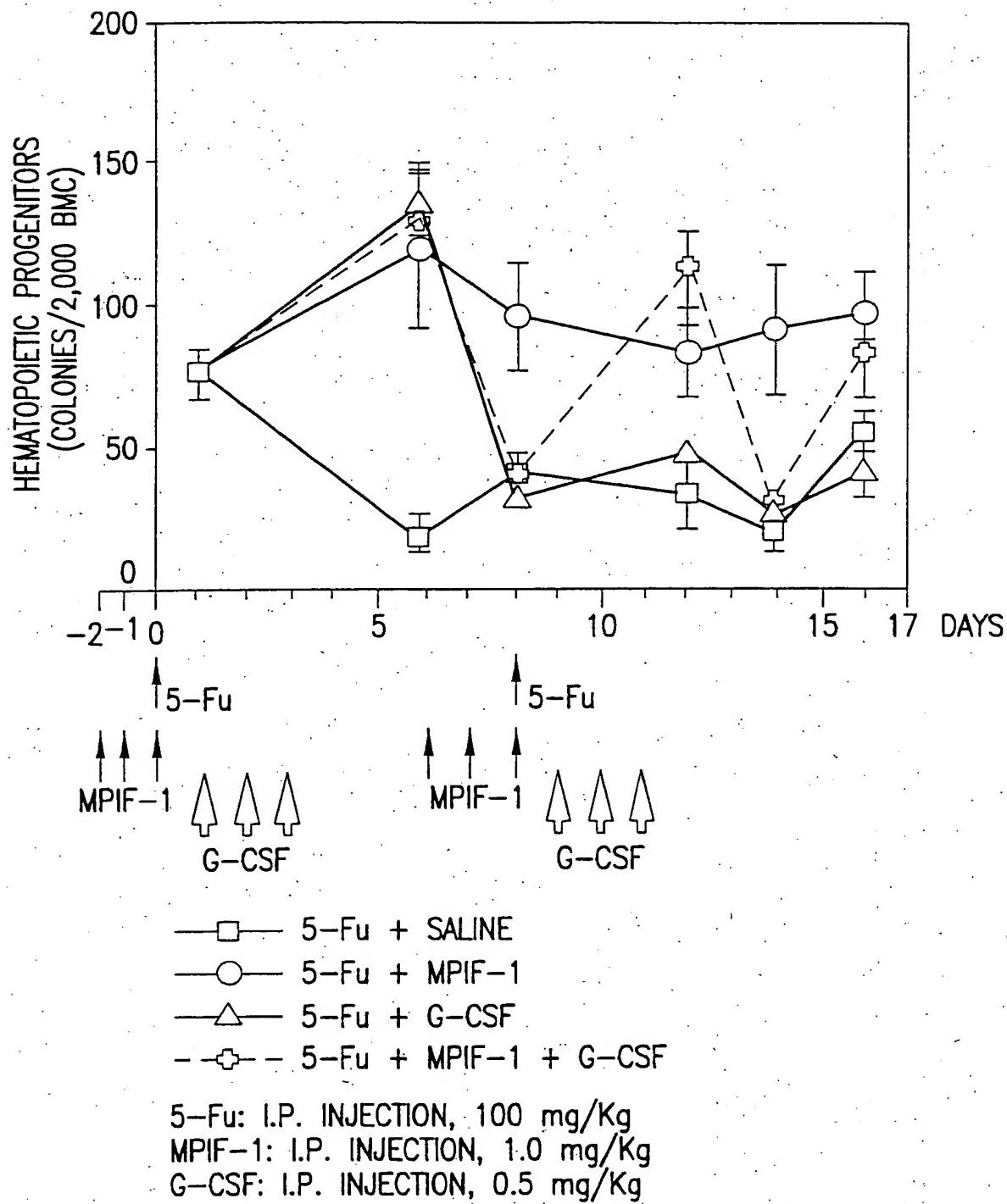


FIG.54

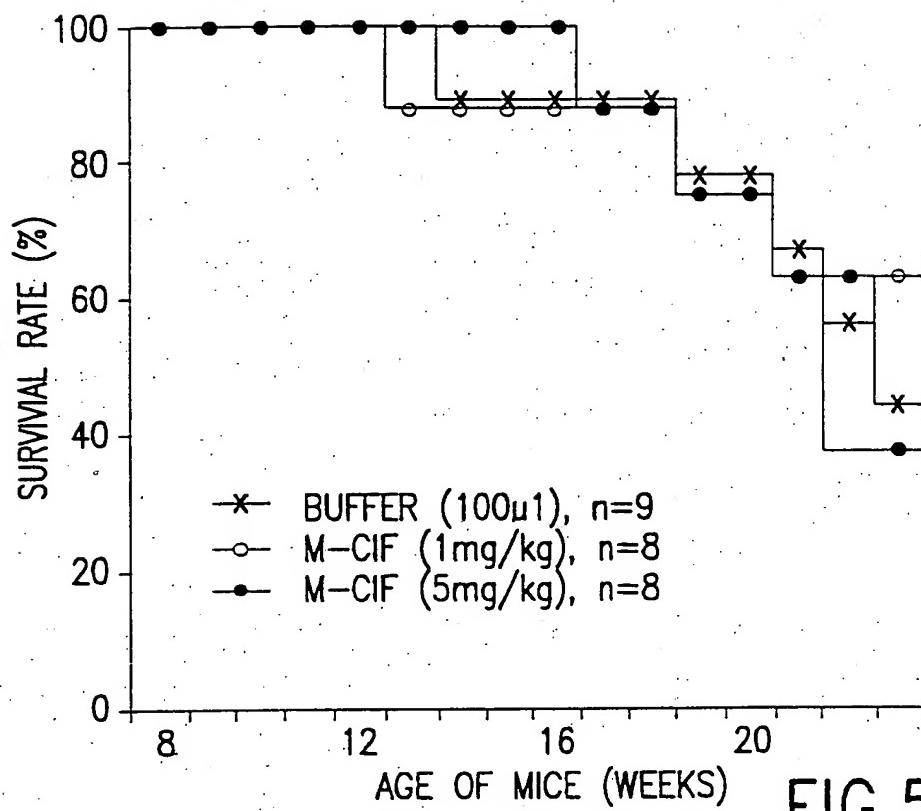


FIG.55

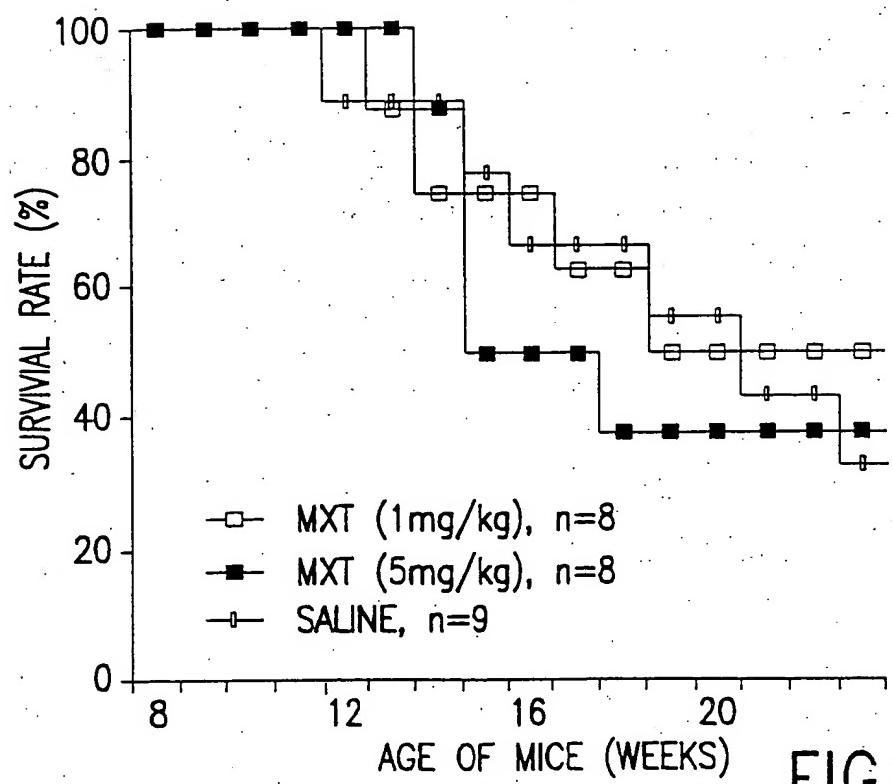


FIG.56

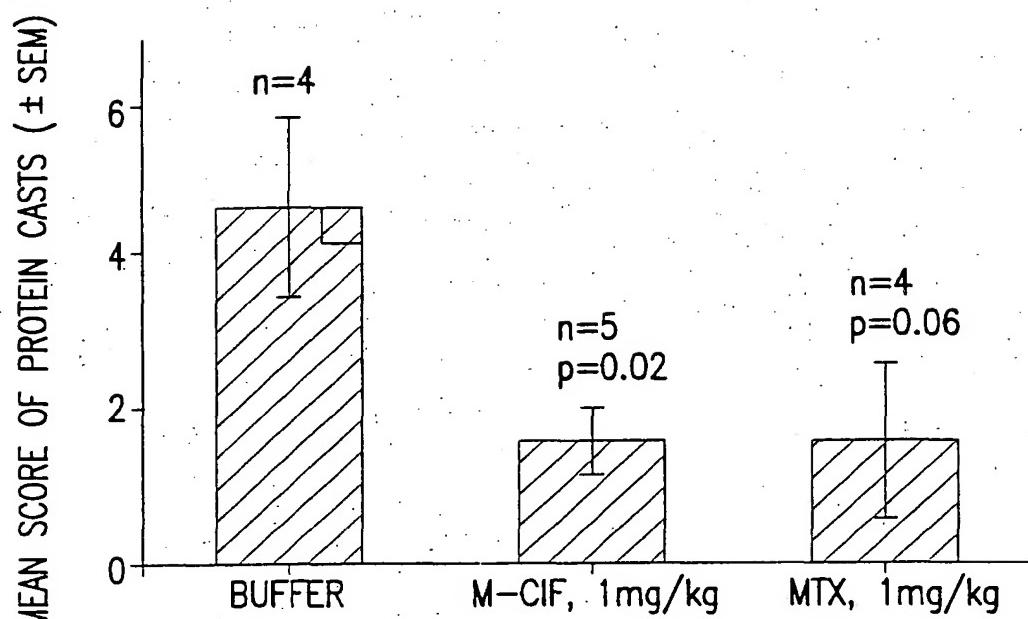


FIG.57

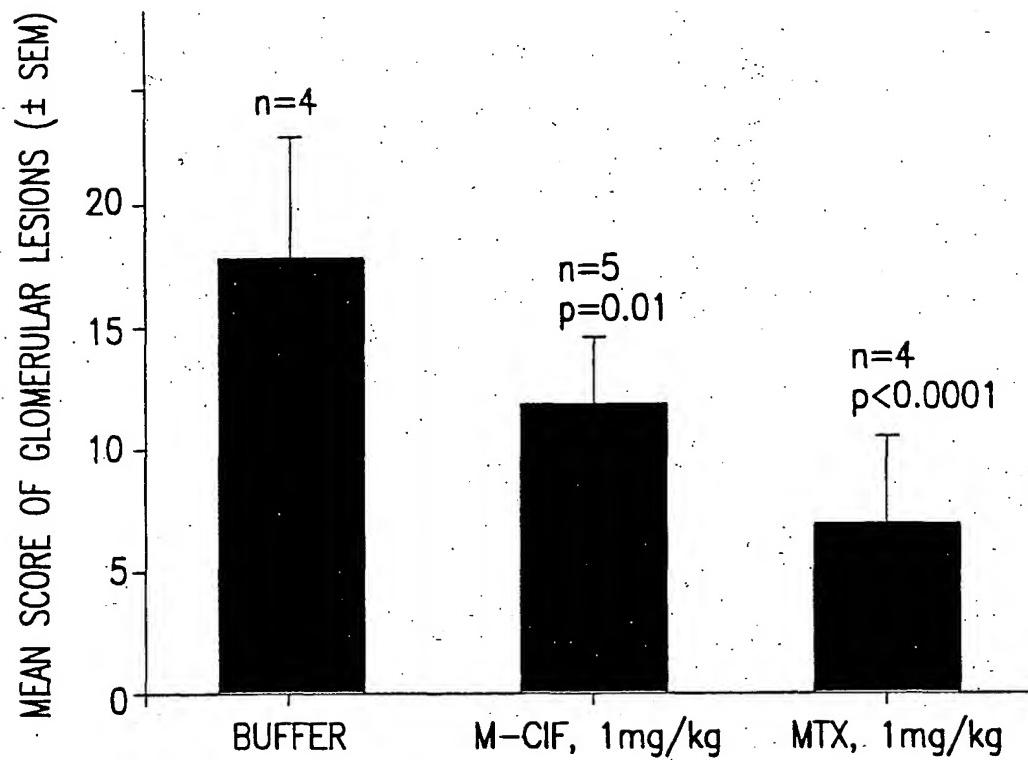


FIG.58

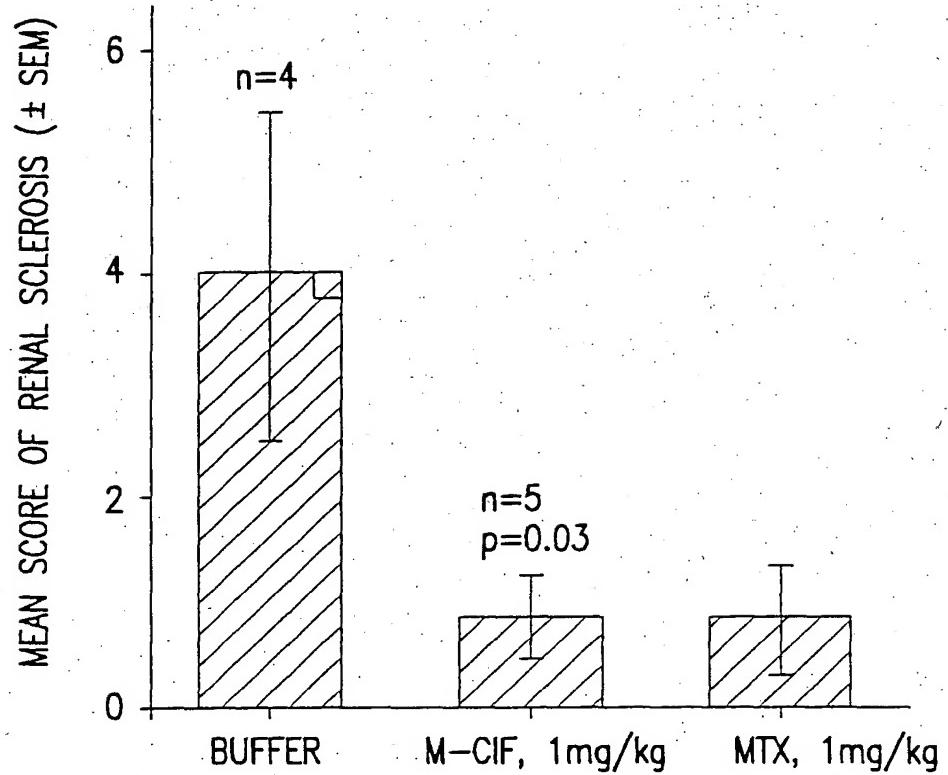


FIG.59

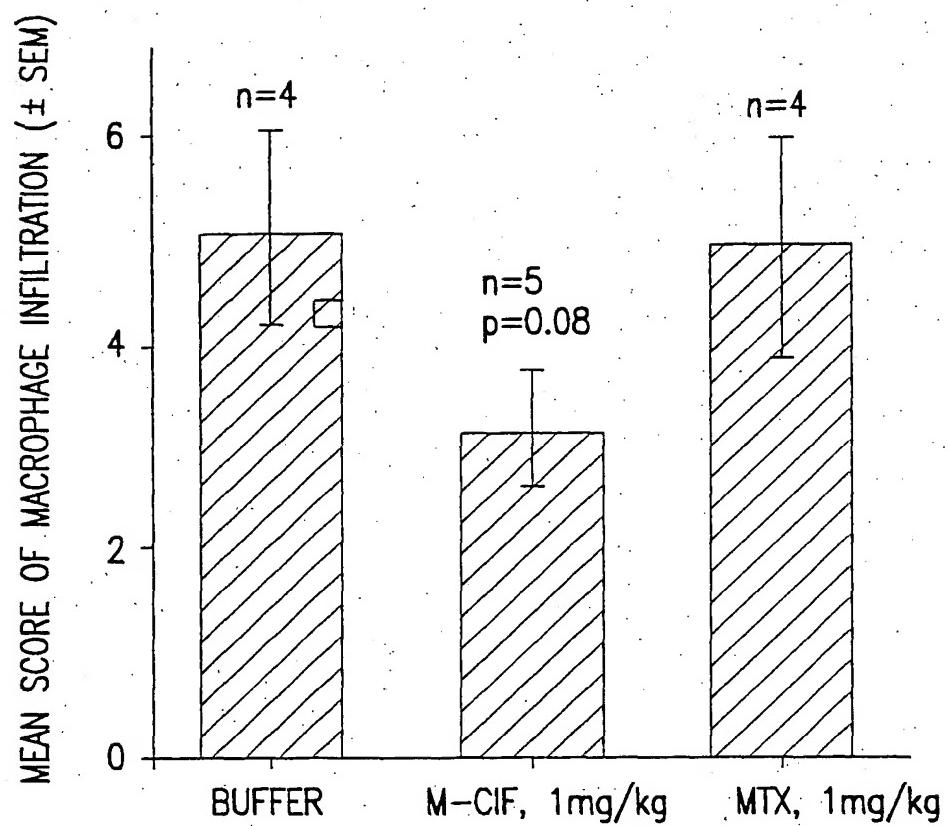


FIG.60

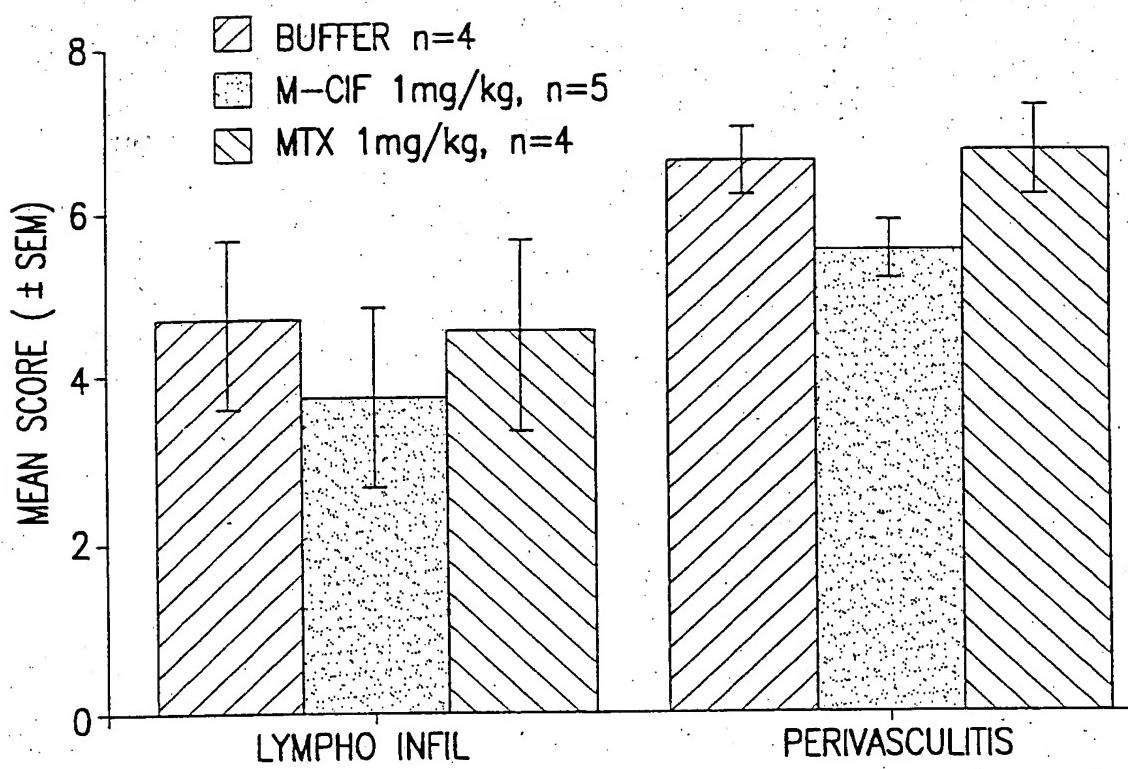


FIG.61

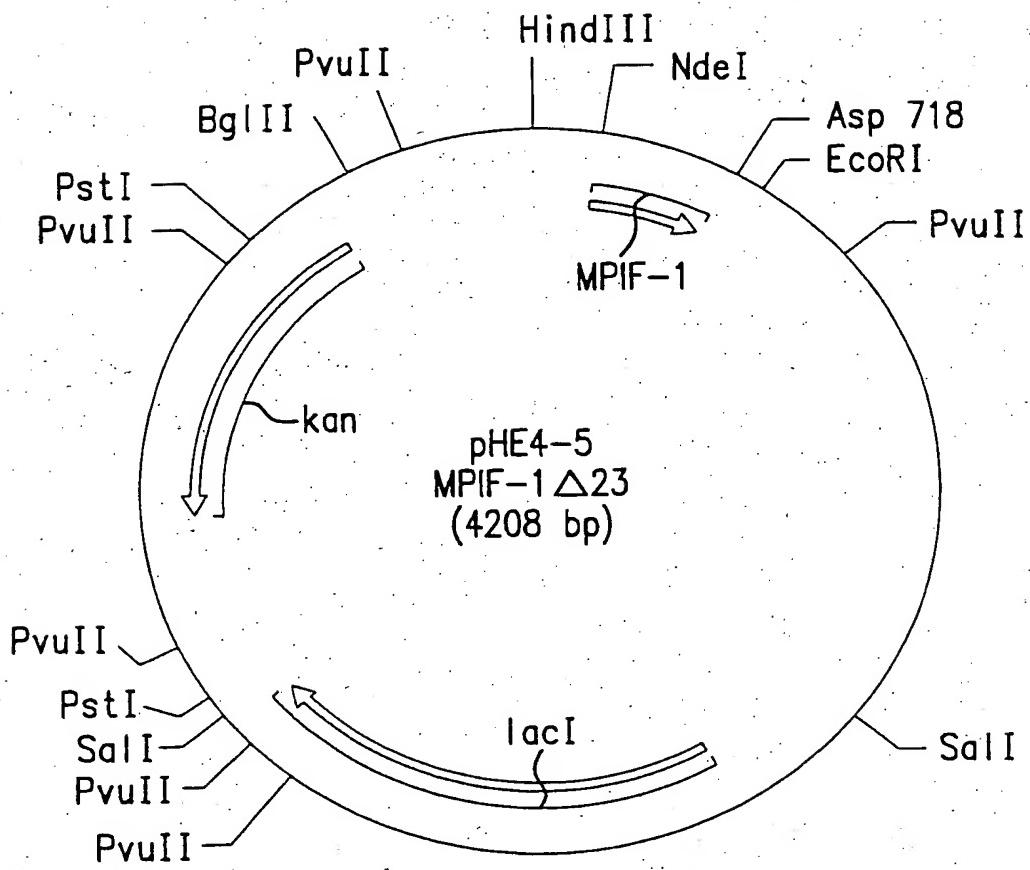


FIG.62

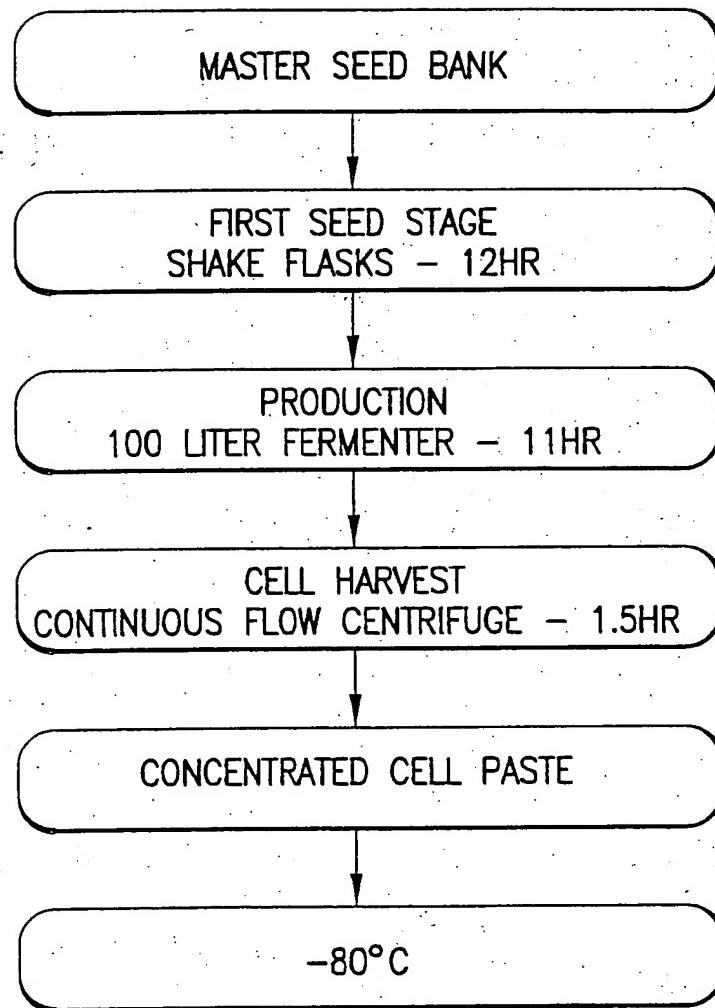


FIG.63

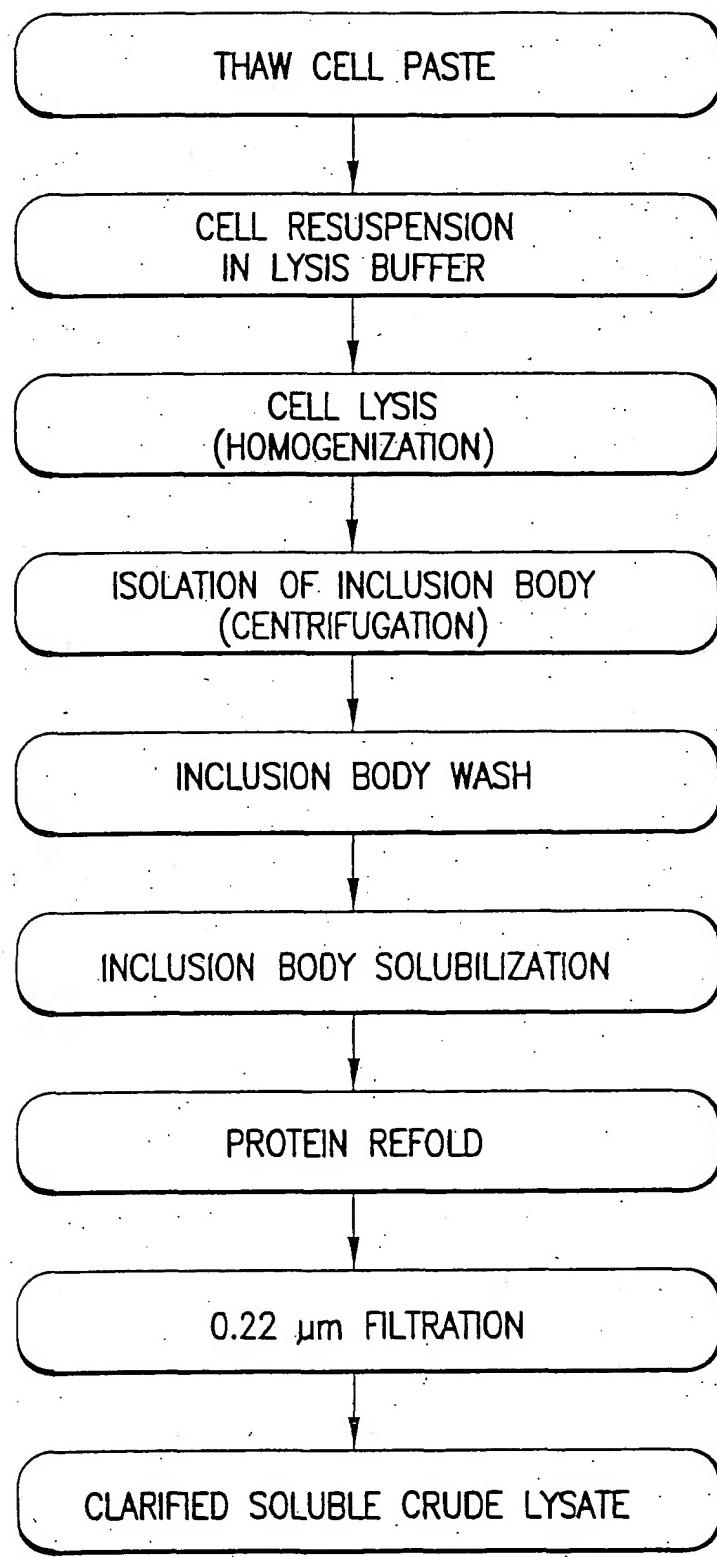


FIG.64

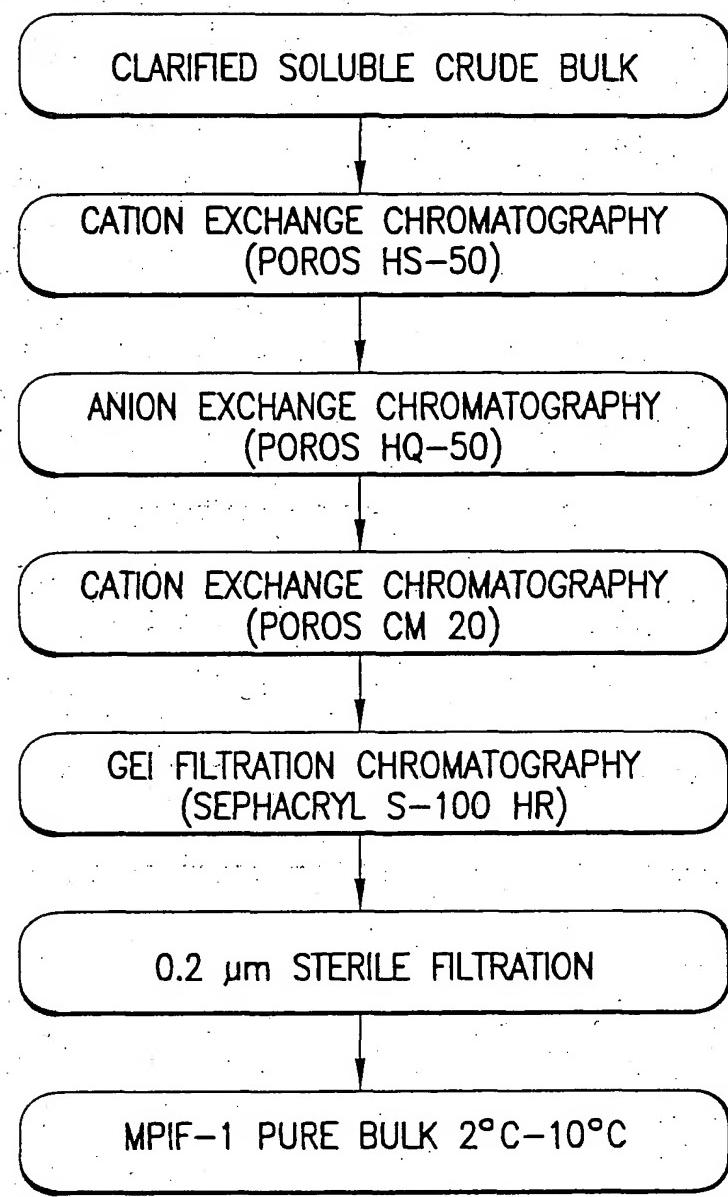


FIG.65

-35

Operator 1

1 AAGCTT AAAAAACTGCAAAAAATAGTTTGACTTG TGAGCGGATAACAAAT

-10

Operator 2

50 TAAGATGTACCCATTG TGAGCGGATAACAAATTTCACACATTAA

S/D

94 AGAGGGAGAAATTACATATG

FIG.66

Hind III

—  
Nde

FIG. 67A

Sequence alignment diagram showing DNA strands A, B, and C. Strand A is the top strand with a poly-A tail. Strand B is the middle strand with a poly-B tail. Strand C is the bottom strand with a poly-C tail. An arrow labeled "oriC" points to a specific sequence in strand C. Reference sequences are shown on the right with numbers 780, 910, 1040, and 1170.

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FIG. 67B

FIG. 67C

# FIG. 67D

AATACTCACTCGCAATTAGCCGAAACGGCAAGCCGACTGAGTGCCATTCGGTTCACAAACCATGCAAATGCTGAATGAGGCCATTCGTTCCCACTGCCATGCGTGCCTGGCAA  
 ++++++ 2340  
 TTATAGCTGAGCTTAGTTAACGCTTACGGTACGCCATGGCTTACGGTACGCCAAAGCTTACGTTAGCAAGGTACCCGTAGCAAGGTAGGCCAACGGTT  
 | ac |  
 K Y L T R N Q I Q P I A E R E G D W S A M S G F Q Q T M Q M L N E G I V P T A M L V A N

---

CGATCAGATGGGGCTGGGGCAATGGGGCAATACGGAGTGGGGTGGGGATACGGTAGTGGGATACGCAATACGGAAAGGAGGCTCACTGTTATACCCGGGTAAACCAACCATC  
 ++++++ 2470  
 GCTAGCTACCGGACCCGGTTACGGGGTAATGGCTCACGGCAACCCGGCAACCCGGCAACCCGGCAACCCGGCAACCCGGCAACCCGGCAACCCGGCAATGGCTGGTAG  
 | ac |  
 D Q M A L G A M R A I T E S C L R V G A D I S V V C Y D D T E D S S C Y I P P L T I I

---

Pvu II  
 AAAAGGGATTTGGCCCTGGGGAAACCCAGGCTGGACCCCTGGCAACTCTCAGGGCCAGGGTGGCAAGGCCAATCAGGCTGGCCCTGGCCCTGGCCCTGGCCCA  
 ++++++ 2600  
 TTGCTCTAAAGGGAGGACCCGGTTGGCTGGGAAAGGAGCTGGAGAGTGGCAACCTGGGACTTCGGGTTAGTCGACAACGGGAGAGTGGACACTTCTTGGGGACCCGGGT  
 | ac |  
 K Q D F R L L G Q T S V D R L L Q L S Q G Q A V K G N Q L L P V S L V K R K T T L A P

---

Pvu II  
 ATACGGCAAACGGCTCTCCCGCGCGTTGGCCGATTCATTAAGGCTGGCACCGACGGTTCCCGACTGGAAAGCCGGCAGTGCGCCAAACGCAAATTAATGTAAGTAAAGCTGGCAATTTGCGACCAAG  
 ++++++ 2730  
 TAGGCTTGGCGAGAGGGCCCAACGGCTAAGTAAATACGTCACCTGGCCGTCACCTGGCTGGGTTAGCTGGGTTAAATACATTCGAATCGGCCCTAACAGCTGGTTTC  
 | ac |  
 N T Q T A S P R A L A D S L M Q L A R Q V S R L E S G Q

FIG. 67E

FIG. 67F

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FIG. 67G